

Information transmission rate in biological signaling pathways

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Summary

Living systems rely on information processing at multiple spatiotemporal scales, from the rapid electrical spiking of neurons to the slower biochemical signaling cascades within and between cells. While neural information transmission has been studied extensively for more than a century, quantification of biochemical signaling capacity has only recently become feasible due to advances in genetic engineering, fluorescent biosensors, and single-cell analysis. This dissertation, consisting of three published papers, investigates the maximum rate of information transmission in biochemical pathways, focusing on the channel capacity of the MAPK/ERK cascade and juxtracrine communication in multicellular systems.

To address this, the first two papers introduce methods for quantifying information transmission rates in systems where the input consists of binary stimulation sequences and the output is a real-valued trajectory of signaling activity measured in single cells. Probabilistic classifiers (k -nearest neighbors and multilayer perceptron) were trained to reconstruct input sequences from short output slices, allowing conditional entropy estimation and thus bitrate calculation.

In collaboration with experimentalists, I showed that the MAPK/ERK pathway is capable of transmitting 6–11 bits per hour, an order of magnitude more than earlier theoretical estimates. The MAPK/ERK pathway was stimulated optogenetically (via optoFGFR) and monitored using a fluorescent ERK activity reporter. The refractory time was found to be ~8–10 minutes, allowing more than five detectable pulses per hour. This confirmed that the pathway is well-suited to mediate multiple concurrent cellular processes requiring transient activity on the timescale of tens of minutes, such as migration, vesicle release, or transcriptional priming. As a side result, a counteracting optoFGFR-PI3K-IP₃-Ca²⁺-calcineurin pathway was analyzed and found to have shorter latency (~2 min) and higher capacity (≥14 bits/h).

In non-small-cell lung carcinoma cells (STE-1) harboring the EML4-ALK gene fusion, the MAPK/ERK pathway showed drastically reduced channel capacity (<2 bits/h), with only ~15% of cells retaining measurable transmission. Treatment with an ALK inhibitor partially restored responsiveness and increased capacity, although not to the level of normal epithelial cells.

The third study expanded the analysis to long-range intercellular communication. Using an agent-based model of a cell monolayer inspired by mechanochemical ERK waves, cell activity was allowed to propagate juxtracrinely across hundreds of cells. Information transmitted by such wavefronts achieved rates of ~0.5 bits/h under physiological conditions. Stochastic effects limiting information transmission: propagation speed variability, front extinction and new front spawning were identified and quantitatively analyzed. The interplay between front extinction (less likely in wide tissues) and front spawning (more likely in wide tissues) revealed that the optimal tissue width for reliable communication was only ~6 cells across.

By introducing experimental methods for bitrate estimation and protocol optimization, the work provides tools for determining the informational limits of diverse signaling systems. Overall, the findings advance the quantitative understanding of information flow in cellular signaling and open the way for systemic-level analysis.

Streszczenie

Przekaz informacji w organizmach żywych odbywa się na wielu poziomach, od szybkich sygnałów elektrycznych w neuronach, przez biochemiczne szlaki sygnałowe, wewnątrz- i międzykomórkowe, po mechanizmy regulacji genetycznej. O ile przepływ informacji w układzie nerwowym został dość dobrze poznany, kwantyfikacja przepustowości (ang. *channel capacity*) ścieżek sygnałowych stała się możliwa dopiero w ostatnim czasie dzięki rozwojowi technik eksperymentalnych umożliwiających pomiar aktywności białek na poziomie pojedynczych komórek. Przeprowadzenie tej kwantyfikacji w kontekście kaskady MAPK/ERK oraz komunikacji jukstakrynowej (sygnalizacja między sąsiadującymi komórkami) w populacji komórek było celem niniejszej rozprawy.

Autor opracował metodę pozwalającą oszacować prędkość przekazu informacji w systemie przetwarzającym sekwencję krótkich pulsów na ciągłą trajektorię aktywności białek. Probabilistyczna rekonstrukcja wejściowej sekwencji oparta o klasyfikatory (*k*-NN i wielowarstwowy perceptron) umożliwiła wyznaczenie entropii *a posteriori*, a w konsekwencji tempa przekazu informacji.

We współpracy z doświadczalnikami pokazałem, iż szlak sygnalizacyjny MAPK/ERK jest w stanie przekazać 6-11 bitów na godzinę, kilkukrotnie więcej niż przewidywał wcześniejszy teoretyczny model. Ścieżkę MAPK/ERK stymulowano optogenetycznie (poprzez receptor optoFGFR), mierząc aktywację białka ERK przy pomocy fluorescencyjnego reportera. Okres refrakcji, po którym możliwa była detekcja kolejnego sygnału, wyznaczono na 8-10 min, przy rozdzielczości czasowej rzędu 1 min. Rezultat ten potwierdza zdolność ścieżki MAPK/ERK do jednoczesnego brania udziału w kilku procesach takich jak migracja, sekrecja pęcherzyków wewnątrzkomórkowych czy inicjacja transkrypcji, odbywających się w skali dziesiątek minut. Oszacowano również tempo (>14 bit/h) oraz opóźnienie (2 min) przekazu informacji w równoległym szlaku optoFGFR-PI3K-IP₃-Ca²⁺-kalcyneuryna, wykazującym działanie przeciwstawne. Wyznaczone tempo przekazu w tej ścieżce okazało się być wyższe, a opóźnienie krótsze niż w przypadku ścieżki MAPK/ERK.

W komórkach niedrobnokomórkowego raka płuc (NSCLC) zawierających onkogenną fuzję genów EML4-ALK (linia STE-1) zaledwie 15% komórek zachowało mierzalną zdolność transmisji informacji, która jednak nie przekraczała 2 bit/h. Zastosowanie inhibitora ALK przywróciło komunikację, jednak do poziomu niższego niż w przypadku nierakowych komórek nabłonkowych.

W trzeciej pracy skwantyfikowano tempo dalekozasięgowego przekazu informacji w populacji komórek. Przy pomocy modelu agentowego stwierdzono, że dla parametrów charakterystycznych dla mechanochemicznych fal aktywności białka ERK, fronty aktywności mogą propagować się na odległość kilkuset komórek, przekazując maksymalnie około 0,5 bit/h. Opisano stochastyczne efekty: zanik frontów, fluktuacje prędkości propagacji oraz zdolność do spontanicznego tworzenia nowych frontów, który to czynnik okazał się być limitujący dla przekazu informacji w populacjach szerszych niż szerokość optymalna (około 6 komórek).

Wprowadzone metody kwantyfikacji przekazu informacji mają zastosowanie ogólne i pozwalają na badanie teorii informacyjnych limitów w różnych systemach. Opisane wyniki poszerzają wiedzę o przepływie informacji w komórce i stanowią krok w kierunku ilościowej analizy na poziomie systemowym.

Preface

As I type this text, my fingers tap on the keyboard and eyes scan the letters appearing on the screen. Visual information is transferred, integrated and analyzed at a great rate and with small latency by neurons in my nervous system to catch the typographic mistake I have just made in the last word and prompt my hands to hit backspace almost instantly. However, not every problem I encounter can be resolved through the swift neural coordination. If I had been writing this manuscript a century earlier with ink and pen, a careless movement of my hand along the paper edge might have resulted in a tear in my finger. Although receptors and neurons would have rapidly alerted my brain to the injury, the nervous system would not be capable of reaching every cell involved in skin repair, and so other means of communication would need to take over. Signaling molecules like cytokines secreted into the blood would recruit lymphocytes and other immune system components; growth factors diffusing in the extracellular space would stimulate epithelial cells proliferation, while mechanochemical waves propagating outward would direct their movement towards the wound. All these signals would have to be correctly perceived by particular cells to evoke proper behavioral response. This computation and decision-making would be done at the level of intracellular biochemical signaling cascades. Upon activation of protein receptors on the cellular membrane, proteins and other signaling molecules would undergo posttranslational modifications (such as phosphorylation, cleavage, complexification), changing their conformation to reveal catalytic domains and pass the signal further towards effector proteins and transcription factors in the nucleus. Negative feedback loops would then inhibit the signaling pathway and prepare it for receiving of a new stimulus.

Information transmission by neurons has long been mechanistically understood, at least on the single-cell level. Spikes of membrane electrical depolarization traveling along the axon guarantee fast and reliable information transmission. The stereotypical shape of a neural spikes has prompted a conclusion that it is not the spike amplitude, but rather the timing of particular pulses, or interim intervals, that encodes the useful message. How the information is encoded remains a matter of debate: initially, spike frequency has been proposed to represent the signal strength in an analogue manner. However, it has soon been noticed that temporal patterns are capable of encoding much more information per unit time, although no clear mechanism for translating input information into a sophisticated spike train has been identified. Recently, evidence increases that in brain decisions may be made on time scales enough for only one spike, implying parallelized information processing using mechanisms such as a race condition between particular neurons. If this proves true, precise timing of particular spikes will become even more important than initially suspected.

While neural computation has been extensively studied for years, both experimentally and theoretically, biochemical signaling has long lacked suitable tools. Techniques like western blotting, immunostaining or ELISA assays could provide information about pathway activity either at a single timepoint, or only on the populational level, and it was not until the popularization genetic engineering in the 2000s that fluorescent FRET-based biosensors and fluorescently tagged proteins allowed for gathering single cell trajectories of protein activity. Since then, it has become evident that while many pathways operate in a continuous mode, in which the outcome is determined by

a slowly changing level of protein activity, others respond in a pulsatile manner, somehow similar to that of spiking neurons.

It remains an open question, how much information a living organism can acquire and process. In the case of the nervous system, upper bounds have been put on information transmission and processing rates, based on the maximum spiking frequency, its temporal resolution as well as the estimated number of neurons and synapses in the body. *In vivo* experiments were also performed to assess repetitiveness of spike patterns occurring in response to identical stimuli. For example, the single H1 neuron in a blowfly's visual cortex was estimated to transfer roughly 80 bits per second about the visual input presented to the fly (Strong et al. 1998). Treating this as a model of one of the 10^6 cells in the human optic nerve, one can conclude that the brain receives no more than 80 Mb visual input per second, i.e. 15 times less than a raw full HD movie and 30 times more than one compressed with a standard codec.

As cognition emerges from the activity of single neurons, cellular "perception" results from the interplay between multiple biochemical signaling pathways. Although operating on the time scales of minute to hours, rather than single milliseconds, they are capable of transmitting crucial information and making informed decisions. Finding a way to quantify the rate of information flow through such systems is an important challenge and the subject of this work. Although the systemic level is currently beyond reach, in this dissertation I present quantification of the information rate in several systems, putting bounds on their "perception" capacity.

Scientific papers constituting the publication cycle

Paper 1

Nałęcz-Jawecki, P.¹, Gagliardi, P.A.², Kochańczyk, M.¹, Dessauges, C.², Pertz, O.², Lipniacki, T.¹.
The MAPK/ERK channel capacity exceeds 6 bit/hour
PLOS Computational Biology 19(5): e1011155 (2024)
<https://doi.org/10.1371/journal.pcbi.1011155>

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Paper 2

Nałęcz-Jawecki, P.*¹, Roth, L.*², Grabowski, F.¹, Li, S.², Kochańczyk, M.¹, Bugaj, L.J.², Lipniacki, T.^{1,3}
Quantifying cancer- and drug-induced changes in Shannon information capacity of RTK signaling
Scientific Reports 15, 39340 (2025)
<https://doi.org/10.1038/s41598-025-23075-y>

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Paper 3

Nałęcz-Jawecki, P.¹, Szyc, P.², Grabowski, F.¹, Kochańczyk, M.¹, Lipniacki T.¹
Information transmission in a cell monolayer: A numerical study
PLOS Computational Biology 21(2): e1012846 (2025)
<https://doi.org/10.1371/journal.pcbi.1012846>

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Other scientific publications by the author

1. **Nałęcz-Jawecki, P.**, Szymańska, P., Kochańczyk, M., Miękisz, J., Lipniacki, T.
Effective reaction rates for diffusion-limited reaction cycles.
J Chem Phys **143**(21):215102 (2015)
<https://doi.org/10.1063/1.4936131>
2. Korwek, Z.*, Tudelska, K.*, **Nałęcz-Jawecki, P.***, Czerkies. M.*, Prus W., Markiewicz, J.,
Kochańczyk, M., Lipniacki, T.,
Importins promote high-frequency NF-κB oscillations increasing information channel capacity
Biol Direct **11**:61 (2016)
<https://doi.org/10.1186/s13062-016-0164-z>
3. **Nałęcz-Jawecki, P.**, Miękisz, J.,
Mean-Potential Law in Evolutionary Games
Phys. Rev. Lett. **120**, 028101 (2017)
<https://doi.org/10.1103/PhysRevLett.120.028101>
4. Białecki, S., **Nałęcz-Jawecki, P.**, Kaźmierczak, B., Lipniacki, T.
Traveling and standing fronts on curved surfaces.
Physica D **401**, 132215 (2020)
<https://doi.org/10.1016/j.physd.2019.132215>
5. Zmyślony, M., Dradrach, K., Haberkow, J., **Nałęcz-Jawecki, P.**, Rogóż, M., Wasylczyk, P.,
Optical Pliers: Micrometer-Scale, Light-Driven Tools Grown on Optical Fibers.
Adv. Mater. **2002779** (2020)
<https://doi.org/10.1002/adma.202002779>
6. Rogóż, M., Dziekan, Z., Dradrach, K., Zmyślony, M., **Nałęcz-Jawecki, P.**, Grabowski, P.,
Fabjanowicz, B., Podgórska, M., Kudzia, A., Wasylczyk, P.
*From Light-Powered Motors, to Micro-Grippers, to Crawling Caterpillars, Snails and Beyond—
Light-Responsive Oriented Polymers in Action.*
Materials, **15**(22), 8214 (2022)
<https://doi.org/10.3390/ma15228214>
7. Grabowski, F., **Nałęcz-Jawecki, P.**, Lipniacki, T.
Predictive power of non-identifiable models.
Sci Rep **13**, 11143 (2023)
<https://doi.org/10.1038/s41598-023-37939-8>

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1 Thesis structure

1.1 Hypotheses & goals

The main goal of this work was to determine the channel capacity, i.e. the maximum information transmission rate, in biochemical signaling pathways.

The study was prompted by an earlier investigation held in the lab (Korwek et al. 2016), in which we showed, how the explicit inclusion of importins in the model of NF- κ B nuclear shuttling shortens the corresponding pathway's refractory time. Consequently, it allows for more frequent occurrence of NF- κ B activity pulses that could then carry information. While the work did not provide rigorous estimates of the information transmission rate, it put forward the idea that biochemical signaling systems may be well-designed for transmitting information encoded in the timing of transient activity pulses.

In this dissertation, I investigated the MAPK/ERK pathway. My advisors and I hypothesized that:

1. Transient activity pulses of the pathway are repetitive enough to encode information sufficient to drive the processes regulated by the pathway,
2. Oncogenic mutations may influence the temporal precision of pulse evocation, and thus alter the cell's ability to transmit pulse-encoded information,
3. In a cell population, transient pathway activity, transferred to neighboring cells by juxtacrine interactions, may form activity waves that can efficiently carry information over long distances across the population.

These hypotheses were addressed in the publication cycle constituting this thesis. Although the experimental work (and thus biological results) concentrated on the MAPK/ERK pathway, the questions and presented methods are generic and can be applied to other pathways in the future.

1.2 The publication cycle

The thesis consists of three scientific papers. Although I co-authored the forementioned preliminary work (Korwek et al. 2016), I decided not to include it in the publication cycle, as I was not the main contributor to that result.

Papers 1 and 2 are closely related and constitute the core of the thesis. Both papers concentrate on experimental, model-free quantification of information transmission rate through the MAPK/ERK pathway (referred to as the RTK/ERK pathway in Paper 2). In Paper 1, a method to quantify the pulse-encoded information flow upon existing experimental limitations was established and the rate was measured in several encoding protocols, imposing a lower bound on the pathway's capacity as a communication channel. In Paper 2, the method was improved to allow for numerical protocol optimization and thus direct channel capacity estimation. It was applied to cells harboring a cancer mutation affecting the pathway to evaluate the mutation's impact on the information capacity, and to verify whether the capacity is restored by a standard drug treatment. As a side result, the information capacity of a parallel signaling pathway, leading through PI3K, IP₃, Ca²⁺, and calcineurin, was estimated (in normal cells).

Paper 3 is a model-oriented work, in which information transmission in tissue was evaluated. Cells forming a monolayer were modeled as agents in the SEIR model (used broadly, e.g., for epidemics modeling) and allowed to propagate their (transient) activity to neighbors. The ability to obtain coherent front propagation in the monolayer, and thus effective information transmission over a long distance, was evaluated. Stochastic phenomena disrupting this process were identified and analyzed. Information transmission rate in a simple protocol family was quantified and sensitivity analysis was performed. Paper 3 may inspire future researchers to measure information through juxtra- and paracrine signaling in the living tissue in processes like collective cell movement or infection.

1.3 Author contribution

As the first two papers include both experimental results and theoretical analysis, they required collaboration of researchers with various insights and skills. The author of this thesis was responsible for the theoretical part, including mathematical method development, experiment design, data analysis and source code development. In the theoretical Paper 3, the author was the main contributor to bitrate estimation procedure and source code development, and is responsible for the quantitative analysis of the model. The authors' contribution statements are appended to this document.

2 Introduction

In this Thesis introduction, I first introduce the topic in a historical perspective to situate the work in a larger context. Then I introduce basic concepts of the work and state key difficulties that were overcome in this thesis. Next, I describe the quantification methods used in the papers and explain differences between them. Finally, I present the biological results and possible implications, as well as summarize the research in the context of the posed hypotheses.

2.1 Measuring information transmission in biological systems

2.1.1 Telegraphy and the neural system

Arguably the earliest milestone in the modern study of information transmission in biological systems was marked by the discovery of action potentials (today known as spikes) in the frog's neuron by Emil du Bois-Reymond in 1840s (du Bois-Reymond 1848). Although the electrical impulses were originally described as “instantaneous”, their propagation speed along the neuron was not infinite. As shown shortly after by Reymond's colleague, Herman von Helmholtz, spikes in that neuron propagate at a relatively low speed of 20 m/s (Helmholtz 1850). Combined with the frog's motoneuron length of several centimeters, this discovery posed the first physiological bound on the latency of information transmission in a living organism.

In general, an information channel is characterized by two major parameters: its *latency* (the delay after which the message is available to the receiver) and *information capacity* (maximum information transmission rate, in bits per unit time). The latter value remained yet to be measured. The first step was done half a century after Helmholtz's experiments, when Francis Gotch and George J. Burch observed that a frog's neuron stimulated with two electrical impulses separated by an interval shorter than a certain threshold (~6 ms, strongly dependent on temperature) did not respond to the latter impulse (Gotch and Burch 1899).

It was soon noticed that a stronger stimulus implied shorter refractory time and thus more frequent action potentials. A simple encoding scheme was proposed, in which the spiking frequency represented the (total) input amplitude. However, in 1930s, analysis of signal summation in dendrites lead Rafael Lorente de Nó to the conclusion that occurrence of a postsynaptic impulse required synchronous input from several synapses at the time scale of several milliseconds (de Nó 1939), i.e. shorter than the refractory time. This implied that not only the spike frequency, but also the exact spike timing could carry useful information.

Meanwhile, development of telegraphy inspired engineers and mathematicians to seek the ultimate bound for the rate at which information (or, as it was initially referred, ‘intelligence’) can be transmitted by a communication device. In 1924, Harry Nyquist pointed out that the information carried by a single symbol is equal to the logarithm of the ‘alphabet’ size (in telegraphy: number of discernible electric currents) (Nyquist 1924). For example, if a system can distinguish three current levels (‘-1’, ‘0’, ‘+1’), the information was encoded in ternary symbols, and the information contained by each such symbol was equivalent to $\log_2 3 \cong 1.6$ binary digits (today called *bits*).

For technological reasons, operation of devices used in telegraphy was usually restricted to a certain band in the frequency domain, therefore such devices could not transmit sharp, spike-like impulses,

nor precisely reflect an instantaneous switch between states. In a 1928 paper, Nyquist showed that a function with (one-sided) Fourier spectrum bounded to an interval of length B (called bandwidth) is uniquely determined by values at timepoints $k/2B$ for integer k (Nyquist 1928). Consequently, $2B$ is the maximum frequency, at which consecutive symbols (currents) can be sent so that they do not interfere with each other. The information transmission rate can therefore reach at most $2B \log_2 n$, where n is the number of distinguishable current levels.

These results were forged into a mathematical formalism by Claude Shannon, who supplied the probability theory with the formal notion of entropy, representing the average information (in bits) gained by learning the result of a random experiment (Shannon 1948). Shannon extended Nyquist's results by allowing arbitrary encoding schemes (not limited to independent symbols). Instead, he assumed that the measurement was disrupted by an additive gaussian noise with a given power spectrum $N(f)$, and concluded that the channel the capacity equals

$$C = \int_0^B \log_2 \left(1 + \frac{S(f)}{N(f)} \right) df \quad (1)$$

where $S(f)$ is the average power spectrum of the meaningful signal. This formula is currently known as Shannon-Hartley theorem.

The novel idea of quantifying information rate was eagerly welcomed by neuroscientists. However, as neurons are highly nonlinear systems, the Shannon-Hartley formula could not render reliable estimates for a neuron's channel capacity, which then required other techniques. It is natural to use a model, in which the neuron's activity is represented by a series of timepoints, in which a spike occurs. The information contained in such a sequence depends on the statistical distribution of spike trains, which must account for physiological constraints such as refractory time or maximum average spike frequency, and on the temporal resolution, i.e., how precisely the timing of the spike can be measured or passed further. The easiest way to encode a binary sequence in a spike train is to use a straightforward (binary) encoding scheme: in regular time slots occurring once per refractory time T_{ref} a spike is initiated if the corresponding digit in the sequence is a '1'. However, such a scheme is neither optimal nor observed in nature. Indeed, if the temporal resolution Δt is finer than T_{ref} , one may allow spikes to occur not only at multiplicities of T_{ref} , but also in more frequent slots, for example $T_{\text{ref}}, (T_{\text{ref}} + \Delta t), (T_{\text{ref}} + 2\Delta t), \dots, 2T_{\text{ref}}$ after the previous spike. Assuming for simplicity that T_{ref} is a multiplicity of Δt , each interval between spikes has $1 + \frac{T_{\text{ref}}}{\Delta t}$ possible lengths, and thus the sequence can encode $\log_2 \left(1 + \frac{T_{\text{ref}}}{\Delta t} \right)$ bits per spike. As the average interval between spikes is $\frac{3}{2} T_{\text{ref}}$, the information transmission rate equals $\frac{2}{3} T_{\text{ref}}^{-1} \log_2 \left(1 + \frac{T_{\text{ref}}}{\Delta t} \right)$, which (for $\frac{T_{\text{ref}}}{\Delta t} > 2\sqrt{2} - 1 \cong 1.8$) results in a rate higher than the 1 bit per T_{ref} . In a model with discrete time quantized by Δt , it can be proved that the information rate is maximal when the intervals between subsequent spikes are statistically independent and given by $T_{\text{ref}} + k\Delta t$, where k is drawn from the geometric distribution $p_k = (1 - q)q^{-k}$, ($k = 0, 1, \dots$) with parameter q dependent on the ratio $\frac{T_{\text{ref}}}{\Delta t}$.

In 1952, Donald MacKay and Warren McCulloch (MacKay and McCulloch 1952) conducted a similar reasoning and evaluated the equations using the shortest experimentally measured refractory time ($T_{\text{ref}} = 1$ ms) and the observed standard deviation of synaptic delay ($\Delta t = 0.05$ ms) as temporal

resolution, obtaining an upper bound for the neuron's channel capacity ($\frac{2}{3} T_{\text{ref}}^{-1} \log_2 \left(1 + \frac{T_{\text{ref}}}{\Delta t} \right) \approx 3000 \text{ bit/s}$, three times more than the $\frac{1 \text{ bit}}{T_{\text{ref}}}$ that could be sent with the straightforward binary encoding scheme). Under an additional physiological constraint for maximum average firing rate (50 Hz), the estimate gave ca. 500 bit/s. (Of note, both refractory time and mean firing rate differ significantly across types of neurons, implying different bounds on the channel capacity.)

While later mathematical works, e.g., (Rapoport and Horvath 1960), improved the formalism, the biological question remained, how the information was encoded in the sequence of pulses, and how efficiently the theoretical channel capacity was utilized in living organisms. These questions are inherently connected: encoding information in temporal-average features, such as mean spiking frequency, implies high redundancy in the signal, and thus relatively low information transmission rate. Therefore, high information content in the spike train would suggest that precise timing of particular pulses was meaningful. This reasoning was followed by William Bialek and his collaborators in the 1990s (Rieke et al. 1993; Strong et al. 1998), who measured the variability of the neural response to a repetitive sensory input. They stimulated living animals (frogs, crickets, and flies) with a continuous sensory input (tactile or visual) and tried to reconstruct it based on the spike train (measured, respectively, in a motoneuron or the motion-sensitive H1 neuron in the fly's visual system). The reconstruction quality was evaluated to establish the information transmission rate, which was found between 75 and 300 bit/s. A parallel measurement of spiking frequency showed that the information rate accounted for 1.8-3.2 bit per spike, supporting the hypothesis that the timing of single spikes is crucial. This was consistent with other works that indicated that the observed delay between input and reaction is long enough only for a one or two spikes (Land and Collett 1974), and therefore the decision is made before 'spiking frequency' could be established (Rieke et al. 1993).

A thorough review of information rate estimations in various neurons is available here in (Borst and Theunissen 1999), Table 2.

2.1.2 Biochemical signaling pathways

Once adrenaline was discovered in 1890s by George Oliver, Edward Schäfer, and others (Olivier and Schäfer 1894), and other hormones shortly after (Bayliss and Starling 1902; Stiles 1917; Butenandt 1929), it became clear that biological information transmission was not restricted to the nervous system. However, the complex intracellular machinery responsible for reception and processing of these extracellular signals remained a mystery until late 1950s when Earl Sutherland and Theodore Rall first uncovered the role of the small-molecule 'secondary messenger' cAMP (Berthet et al. 1957; Rall and Sutherland 1958). Further study revealed multiple signaling pathways, including the NF- κ B (Sen and Baltimore 1986) and MAPK/ERK pathways (multiple papers between 1986-1993, reviewed in (Ahn 1993)).

A teleological question arose, why these highly conserved pathways have a multi-step cascade form. As one of its crucial functions, the requirement of signal thresholding was pointed out – due to multiple non-linear steps, the signal-response curve gains a relatively sharp sigmoidal shape. Consequently, the downstream components operate in a switch-like manner and thus can distinguish roughly two input states (below or above the threshold), i.e., contain one bit of

information on the stimulus. This hypothesis was addressed in 2011 by Andre Levchenko and collaborators (Cheong et al. 2011), who measured the activity of NF- κ B, ATF-2, Erk and Ca²⁺ in response to, respectively, TNF- α , PDGF, EGF and UDP, and showed that the mutual information between the stimulant concentration and activity of the central agent (protein or ion) of the respective pathway at a given timepoint post stimulation was in all cases between 0.6 and 1.2 bit. Three years later, similar results were obtained for different pathways by a collaboration led by Roy Wollman (Selimkhanov et al. 2014), who showed that the full time course of the activity of the central agent (ERK, Ca²⁺, or NF- κ B) contained only slightly more information (<2 bits) about the concentration of the stimulant (EGF, ATP and LPS, respectively). Recent studies investigated the sources of variability in the response and pointed that the information loss, as observed by an external observer, is largely due to phenotypic differences between cells. In (Kramar et al. 2025), the authors employed a genetically modified version (optoFGFR) of a growth factor receptor (FGFR) that could be stimulated with light. They stimulated the cells repetitively with various light doses to show that the ERK response in individual cells is repetitive enough to allow for distinction of at least 4 stimulation levels (corresponding to 2 bits of mutual information between the light dose and reporter readout).

Probably due to the lack of suitable experimental techniques, the problem of estimating the information transmission rate in biochemical pathways received much less attention. In 2010, Pieter Rein ten Wolde's group (de Ronde et al. 2010) analyzed theoretically the spectrum of gain-to-noise ratio, and thus the information rate through the Shannon-Hartley linear formula, in several feedback schemes. In a 2016 paper (Korwek et al. 2016), we analyzed how often pulses of NF- κ B activity can be evoked, exploring the idea that pulses are crucial carriers of information transmission in biochemical systems and providing an initial estimate for the channel capacity of the pathway. Three years later, (Grabowski et al. 2019) provided a model-based estimate of the information transmission rate through the MAPK/ERK pathway. In a recent paper, Henry Mattingly and collaborators (Mattingly et al. 2021) experimentally measured information transmission rate through the *E. coli* chemotactic pathway (from MeAsp gradient to CheA activity) and showed that – in the linear response regime – it scales quadratically with the gradient and reaches around 2 bit/min for the gradient of 1.5-fold change per mm. This was twice more than necessary to explain the observed bacterial up-gradient motion using a tumble-and-run model.

A thorough review of information and information transmission rate quantification in biochemical signaling pathways can be found here (Tang and Hoffmann 2022).

3 Mathematical formulation of the problem

3.1 Basic notions

Information (or *entropy*) of a given random variable X with distribution p is defined as

$$H(X) = \mathbb{E} \log p(X) \quad (2)$$

where p is its probability mass function (if X is discrete) or probability density function (for continuous X). Entropy is subadditive, i.e., for any two random variables X_1, X_2 the joint entropy of the pair satisfies $H(X_1, X_2) \leq H(X_1) + H(X_2)$; equality holds if X_1 and X_2 are independent. If X is an infinitely long random process, its entropy is typically infinite; however, for discrete time processes one can speak of *average information* (or *entropy*) *per symbol*:

$$H^s(X) = \lim_{N \rightarrow \infty} \frac{1}{N} H(X_1, \dots, X_N) \quad (3)$$

Here, $H(X_1, \dots, X_N)$ is the entropy of the joint variable $(X_1, \dots, X_N)$. If particular symbols X_i are independent identically distributed variables, then $H^s(X) = H(X_i)$. If X_i are identically distributed, but not independent, then $0 \leq H^s(X) \leq H(X_i)$, as there is some information redundancy in the sequence. If symbols are sent in regular slots with intervals ΔT , the *information* (*entropy*) *rate* (per unit time) is

$$h(X) = \frac{1}{\Delta T} H^s(X) \quad (4)$$

Mutual information between two random variables X and Y determines how much the entropy (uncertainty) of the first variable is reduced when the value of the second variable is known:

$$I(X; Y) = H(X) - H(X|Y) \quad (5)$$

Here, $H(X|Y)$ is the conditional entropy, i.e. expected entropy of the variable X conditioned on the value of Y . It can be proved that the mutual information is symmetric, i.e., $I(X; Y) = I(Y; X)$. Again, when X and Y are temporal sequences, the useful quantities are *average mutual information per symbol*

$$I^s(X; Y) = \lim_{N \rightarrow \infty} \frac{1}{N} I(X_1, \dots, X_N; Y_1, \dots, Y_N) \quad (6)$$

and *information transmission rate* per unit time (or shortly *bitrate*)

$$b(X; Y) = \frac{1}{\Delta T} I^s(X; Y) \quad (7)$$

When $X \rightarrow Y$ is a communication channel, i.e., Y is determined by X (and a stochastic factor), one can speak of the *channel capacity*, i.e., bitrate optimized over all possible distributions of X :

$$CC = \sup_x [b(X; Y)]_{X \sim x} \quad (8)$$

3.2 Biological systems as communication channels

In this thesis, I treated a biological signaling pathway as a communication channel, which transforms stimulatory input X into a measurable output Y . In Papers 1 and 2, an optogenetic receptor (optoFGFR) was stimulated with light (proxy of extracellular stimulation) and the activity of a downstream molecule (ERK) was measured using a reporter protein (ERK-KTR) that migrates from the nucleus to the cytoplasm upon activation by ERK (see Paper 1, Fig. 1B for the pathway scheme). Earlier studies showed that the MAPK/ERK pathway responds in a roughly all-or-nothing manner, depending on whether the stimulus strength exceeds a certain threshold (Cheong et al. 2011; Selimkhanov et al. 2014; Ferrell and Machleder 1998). In our experiments the cells were stimulated with short light stimuli that were strong enough to evoke full response in most cells. With optogenetic stimulation at the time scale of seconds or below, the response is governed by the cumulative light dose rather than the stimulation intensity and duration separately, as shown in Figure 2 in (Dessauges et al. 2022). The input was then a sequence of 0-1 digits determining whether the stimulation took place in a given timepoint, while the output was the trajectory (a sequence of real-valued measurements) of ERK-KTR nuclear intensity in single cells.

In the purely theoretical Paper 3, using a spatial kinetic Monte Carlo simulation, we investigated signal transmission in a 2D layer of cells placed in an elongated rectangular reactor. We ‘stimulated’ all cells at one edge of the reactor at certain timepoints and observed the activity of cells at the far end. The fraction of active cells in the last cell layer formed a time series; although the number of cells in the last layer was finite, implying finite granularity of the output, we viewed it as real-valued trajectory for generality and alignment with other papers.

Despite the ongoing research on estimation of mutual information (Paninski 2003; Kraskov et al. 2004; Gao et al. 2017; Czyż et al. 2023), to the best of my knowledge, no general method is known to estimate information rate in the case of the “binary sequence $\rightarrow$ real-valued trajectory” pattern. In the neuroscience context, the real-valued output can easily be converted into a delta-like pattern by determining timepoints at which a spike occurred based on elementary features like the spike onset or peak (Cessac et al. 2010). Mutual information between binary sequences can then be estimated, e.g., with a gaussian delay model or discrete-time approximations. We followed this approach in Paper 3, where the intervals between subsequent activity fronts propagating in the monolayer were long compared to the time in which a single cell remained active, and thus arrivals of particular fronts at the reactor far end could easily be separated. However, this method was not adequate in the studies of the ERK pathway in single cells (Papers 1-2), as the refractory time of the MAPK/ERK pathway is comparable with the reporter recovery time, causing the response pulses to overlap. This posed a technical challenge which I addressed in the papers.

3.3 Protocol optimization

As mentioned, we restricted the stimulation patterns to short, identical impulses delivered according to a binary sequence. In such systems, the information must be carried by the timing of particular pulses, or intervals between them. We did not have a possibility to check how much information *is* transmitted by the systems in a natural setting (e.g., *in vivo*), therefore, we addressed a general question of the maximum rate of information that *can be* transmitted, that is estimating the

pathway's channel capacity (*in vitro*). This required finding the *optimal encoding protocol*, or, more precisely, the optimal distribution of input sequences.

As mentioned in Section 2.1.1, if all stimulation pulses occurring at least T_{ref} after the preceding pulse were perfectly transmitted with resolution Δt , the maximum bitrate would be achieved for intervals drawn independently from

$$T_{\text{ref}} + \Delta t \cdot \text{Geom}(q) \quad (9)$$

where the optimal q depends solely on $\frac{T_{\text{ref}}}{\Delta t}$ and can be obtained numerically. In a real situation the temporal accuracy Δt is not perfect and the refractory time T_{ref} is not a sharp threshold. Moreover, some pulses can be lost in the course of transmission, and spontaneous pulses can be observed in the output. Therefore, while it could be presumed that the optimal protocol should resemble the one given by Eq. (9), the actual optimization had to be based on experimental data.

Due to experimental constraints discussed in the next section, we assumed that the chance for proper pulse transmission depends only on the time from the previous pulse and thus we restricted the analysis to the protocols in which subsequent intervals were drawn independently from a common distribution. In Paper 1, we analyzed three families of interval distributions based on Eq. (9) that were natural candidates to contain the optimal one, and found a common maximum bitrate. The bitrate estimation technique was improved in Paper 2 and allowed us to find the optimal protocol by numerical optimization.

In Paper 3, we did not perform full protocol optimization, but stucked to the straightforward (binary) encoding scheme as described in Section 2.1.1 (the straightforward model is equivalent to Eq. (9) with $\Delta t = T_{\text{ref}} = \Delta T$ and $q = \frac{1}{2}$), and optimized only the symbol frequency ΔT .

3.4 Dataset structure

The refractory time of the MAPK/ERK pathway is 10-20 min, and due to limited cell viability under intensive stimulation, the maximum duration of a single experiment is around 24 h. This allows for accommodating up to around 100 pulses that could potentially evoke a detectable response. This is far less than in classic neuroscience experiments on flies, where a 30-second stimulation renders around 1500 spikes, and can be repeated multiple times. Due to this constraint, the number of different interval lengths that can appear in the input sequence is limited, as each tested interval must occur several times in the sequence to avoid overfitting. Moreover, sequences of two or more consecutive intervals cannot be sampled properly, which prevents bitrate estimation techniques based on “words” of increasing length (Strong et al. 1998). On the other side, fluorescent microscopy allows for parallel measurement of ERK-KTR translocation in hundreds of cells, producing large amounts of (correlated) data. This illustrates the need for new experiment design and data analysis approaches, that were subject and achievement of this thesis.

4 Methods overview

The goal was to estimate bitrate between the binary sequence X and the real-valued output sequence Y . The procedure was designed as follows:

1. Protocol selection and sampling
2. Experiments and quantification
3. Bitrate estimation
 - a. Probabilistic reconstruction of X based on Y
 - b. Computation of bitrate between the reconstruction and true input
4. Encoding protocol optimization

4.1 Protocol selection and sampling

We chose an input interval distribution and randomly drew or randomized the input sequence. Randomization facilitated proper sampling of the interval distribution by ensuring that the number of occurrences of a particular interval length was proportional to the assumed distribution. The intervals were then shuffled (Paper 1) or ordered in a designed way (Paper 2). The proper ordering was crucial for a fair assessment of which intervals should occur in an optimal encoding, as is illustrated by a following example.

Assume that the refractory time of a system is 10 min, i.e., stimulation pulses that occur less than 10 minutes after the previous pulse are ignored (but only if the previous pulse evoked a response). Suppose that each 5-min interval in the stimulatory sequence occurs just after an 8-min interval, while all 7-min intervals are preceded by long intervals (>10 min). Due to refractory time, all pulses after the 7-min and 8-min intervals would be ignored. However, pulses after the 5-min intervals would evoke a full response as if after a 13-min interval, because they would follow an ignored pulse. The chosen interval ordering assured that intervals of given length follow both short and long intervals.

In the theoretical Paper 3, the numerical ‘experiment’ length was not a limiting factor, we used long, randomly generated sequences.

4.2 Experiments and quantifications

The experiments for Papers 1 and 2 were conducted by partner research groups (Pertz Lab, Universität Bern and Bugaj Lab, University of Pennsylvania) according to the protocol described above. Images were acquired with 1-minute resolution and quantified to obtain single-cell trajectories. Simulations for Paper 3 were performed using software developed by other co-authors of the paper. This stage, described in detail in the papers, is not the contribution of the author of the thesis and therefore will not be covered here.

4.3 Bitrate estimation

As mentioned before, direct estimation of bitrate between a binary input sequence X and a real-valued output sequence Y is a hard task. We avoided it by constructing a (probabilistic) reconstruction of the input sequence using simple machine learning algorithms, and computing the bitrate between the input and its reconstruction. The reconstruction was a sequence of the same

length as the input. Depending on the method, particular reconstruction entries contained either a 0/1 digit, being a prediction based on the measured output of whether a stimulation pulse occurred in a given slot, or a real number determining the probability of a ‘1’. Although the probabilistic reconstruction was technically still a series of real values, the bitrate computation was much easier because it could be conducted pointwise, as described further in detail. Due to the Information Processing Inequality, the obtained value was a lower bound on bitrate between X and Y .

4.3.1 Input reconstruction

The core idea of the thesis was to reconstruct the input sequence based on a short slice (6-8 timepoints) of a single cell’s output trajectory. The probability whether a pulse occurred at timepoint t was predicted based on the rolling window $(t + \tau_1, \dots, t + \tau_2)$. Multiple values of τ_1 and τ_2 were tested, but eventually $(\tau_1, \tau_2) = (-1 \text{ min}, 4 \text{ min})$ were used in Paper 1 and $(\tau_1, \tau_2) = (0 \text{ min}, 6 \text{ min})$ in Paper 2. Such slices were long enough to provide reliable predictions, and short enough to prevent precise localization of the timepoint within the experiment.

	Paper 1 (binary reconstruction)	Paper 1 (probabilistic reconstruction)	Paper 2	Paper 3
Used classifier	k -NN	k -NN	MLP	peak detection
Slice position relative to target timepoint	$[-1, 4]$	$[-2, 5]$	$[0, 6]$	$[-1, 1]$
Additional features provided to the classifier	-	-	○ Average cell responsiveness ○ Time from previous pulse	-
Classifier’s output	binary reconstruction	probabilistic reconstruction	probabilistic reconstruction	binary output sequence
Post-processing	consecutive 1’s removed	-	prior probability included	proximate 1’s removed

Box 1. Comparison of binarization algorithms used in Papers 1-3

The reconstruction approaches used across the Papers differed in terms of the used classifier, slice of output supplied to the classifier as well as applied post-processing operations.

Paper 1 (binary reconstruction) and Paper 3 approaches resulted in a sequence of binary digits, with 1’s corresponding to time points in which a stimulation pulse was predicted to have occurred. If two 1’s appeared close to each other in a single reconstruction sequence, one of them was removed to avoid double detection of a single pulse. In Paper 1 (probabilistic reconstruction approach), double detections had milder effect, as the predicted probability of pulse occurrence was typically smoothed around the most probable time point. In Paper 2, the impact of double detections was reduced to the true uncertainty about pulse timing by the inclusion of prior pulse probability based on the time from the previous (not necessarily detected) pulse.

In Paper 1, to predict the pulse probability based on the trajectory slice, we used the k -nearest-neighbor classifier (k -NN; applied to the discrete derivative of the trajectory to eliminate the offset related to reporter expression). In Paper 2, a small neural network (3-layer perceptron; MLP) was used instead. While both k -NN and MLP made good and reliable predictions, MLP was capable of accepting auxiliary variables, such as the time from the previous pulse, typical responsiveness of the

particular cell, or information about the type of treatment applied to the cells, which facilitated generalization. It also came with several technical advantages; most importantly, it was more memory-efficient, which allowed training on a large dataset originating from several experimental replicates.

In Paper 3, binarization was much easier, as the pulses were well separated in time. We used a standard peak detection procedure, with thresholds on minimum peak magnitude and a minimum distance between peaks. As a result, we obtained a timeline of front arrivals at the far end of the reactor, which was then aligned with the input sequence using the previously measured average front velocity.

The methods are summarized in Box 1.

4.3.2 Computation of bitrate

Information transmission rate $b(X; Y)$ is the difference between the input entropy rate $h(X)$ and the conditional entropy rate $h(X|Y)$. The former depends solely on the assumed input distribution, and as such could be computed directly. The latter depends on the channel performance, so had to be established experimentally.

In Paper 1, we estimated the conditional entropy in two manners. In the first approach ('binary reconstruction', or 'reconstruction-based approach', as it was referred to in the paper), we evaluated the classifier performance by constructing the confusion matrix. This gave us the average probability that a pulse actually occurred in a particular slot, provided that it was predicted by the classifier, and vice versa. Computing the entropy of this conditional distribution and averaging over all timepoints completed the goal. As the classifier often predicted pulse occurrence one timepoint too early or too late, a version of this method was also used, in which the conditional distribution was conditioned on three consecutive digits of the reconstruction, rather than a single one. In this version, a 8×2 confusion matrix was used instead of the standard 2×2 one, to account for all possible reconstruction triples.

The second approach in Paper 1 is referred to as 'reconstruction-free approach' in the paper, although with hindsight I suppose 'probabilistic reconstruction approach' would be more accurate, as it relies on the probability of pulse occurrence in particular timepoints, as predicted by the k -NN classifier. We assumed that the classifier was a Bayes classifier, i.e., its predictions p_i correctly represented the conditional probability $\mathbb{P}(X_i = 1|Y)$ of whether a pulse occurred at a given timepoint or not, given the measured response. The conditional entropy was computed directly based on these predictions using the standard formula for a Bernoulli trial

$$H(X_i|Y) = H_B(p_i) = -(p_i \log_2 p_i + (1 - p_i) \log_2 (1 - p_i)) + \text{MM} \quad (10)$$

where MM is the Miller-Madow bias correction term (Miller 1955; Paninski 2003), and averaged over all timepoints i .

In Paper 2, we improved the latter method by replacing the above formula with cross entropy of predictions against the true input sequence:

$$\langle H(X_i|Y) \rangle \leq \langle X_i \cdot p_i + (1 - X_i)(1 - p_i) \rangle \quad (11)$$

The prediction p_i is a function of Y . The cross entropy between X and an arbitrary function $f(Y)$ is an upper bound on the conditional entropy $H(X_i|Y)$, with equality holding iff $f(Y) = \mathbb{P}(X_i = 1|Y)$. Therefore, while for a Bayes classifier this alteration should not have changed the estimate, it guaranteed that the resulting bitrate estimate remained a lower bound even if the assumption on the classifier did not hold.

Both methods in Paper 1 contained an averaging step based on the relation

$$h(X|Y) \leq \frac{1}{\Delta T} \langle H(X_i|Y) \rangle \quad (12)$$

where ΔT is the time interval between consecutive symbols X_i and X_{i+1} . While the inequality is always true (due to Information Processing Inequality), equality holds only for protocols where particular symbols X_i are independent (conditioned on Y). This assumption holds or nearly holds if X_i is a Poisson process, i.e., when interval lengths follow the geometric distribution. However, in general, the right-hand side can substantially overestimate the conditional entropy rate. To eliminate the effect, instead of Ineq. (12) one can use the exact formula that accounts for the prior knowledge on X_i based on earlier stimulation slots:

$$h(X|Y) = \frac{1}{\Delta T} \langle H(X_i|Y, X_1, \dots, X_{i-1}) \rangle \quad (13)$$

This was done in Paper 2 (credits: Frederic Grabowski) by separating prior knowledge (based on the assumed distribution of input sequences) and information gained by observing the resulting trajectory. The MLP was trained to predict the (logit) Bayesian update that was added to the (logit) prior probability of pulse occurrence given the time since the previous stimulatory pulse to give the final prediction. Moreover, the MLP was informed about the time from previous stimulus, and could use this information to know what shape of response to expect. These two improvements resulted in a tighter bound, as the averaging over timepoints did not introduce further loss (Box 2).

	Paper 1 (binary reconstruction)	Paper 1 (probabilistic reconstruction)	Paper 2
Classifier's output	0 or 1 (rounded p_i)	p_i	logit p_i – logit p_{prior}
Final estimator of $\mathbb{P}(X_i = 1 Y)$	Classifier's output adjusted based on the empirical classifier's reliability: $\mathbb{P}(X_i = 1 p_i) = \begin{cases} \frac{TP}{TP+FP} \text{ if round}(p_i) = 1 \\ \frac{FN}{TN+FN} \text{ if round}(p_i) = 0 \end{cases}$ (*)	Classifier's output: $\mathbb{P}(X_i = 1 Y) = p_i$	Classifier's output with prior term: $\mathbb{P}(X_i = 1 Y, Last_i) = p_i$
Conditional entropy estimation	Bernoulli: $H(X_i Y) = H_B(\mathbb{P}(X_i = 1 p_i))$	Bernoulli: $H(X_i Y) = H_B(p_i) + \text{bias correction}$	Cross-entropy: $\langle H(X_i Y, X_1, \dots, X_{i-1}) \rangle \leq -\langle X_i \log_2 p_i + (1 - X_i) \log_2 (1 - p_i) \rangle$
Bitrate computation	$h(X Y) \leq \frac{1}{\Delta T} \langle H(X_i Y) \rangle$	$h(X Y) \leq \frac{1}{\Delta T} \langle H(X_i Y) \rangle$	$h(X Y) = \frac{1}{\Delta T} \langle H(X_i Y, X_1, \dots, X_{i-1}) \rangle$

Box 2. Steps of conditional entropy estimation in different approaches used in Papers 1 and 2.

TP, FP, FN and TN: terms of the confusion matrix (true positives/false positives/false negatives/true negatives), defined as $TP = \mathbb{P}(X_i = 1 \wedge \text{round}(p_i) = 1)$ etc.; H_B : entropy of the Bernoulli distribution, $H_B(p) := -(p \log_2 p + (1 - p) \log_2 (1 - p))$. ΔT : interval between measurement timepoint. Red background: steps introducing significant overestimation of conditional entropy; green background: exact steps (no overestimation); yellow background: steps that theoretically could introduce overestimation, which was likely negligible in our setting; purple: step that can introduce over- or underestimation if classifier is not Bayes.

(*) In Paper 1, to account for the fact that the classifier often indicated a pulse one timepoint before or after its actual timing, we also used a variant version of the binary-reconstruction approach, where the final estimation of $\mathbb{P}(X_i = 1|Y)$ was based on the $\text{round}(p_i)$ values for three subsequent timepoint points: $i - 1$, i and $i + 1$, which formed a binary triple with 8 possible values. The estimate $\mathbb{P}(X_i = 1|p_{i-1}(Y), p_i(Y), p_{i+1}(Y))$ was then established based on a 2×8 ‘confusion matrix’ instead of the standard 2×2 .

In the numerical Paper 3, the output was measured with much finer temporal resolution Δt than the interval ΔT between slots in which stimulation could occur. This allowed us not only to determine whether a front reached the far end of the reactor within a particular time slot, but also to record its exact arrival time with respect to that slot. We established the distribution of the arrival (or two arrivals) closest to a particular time point $t + T_{\text{transit}}$ (where T_{transit} denotes the average time the fronts required to reach the far end the reactor) conditioned on whether a front was initialized at t . Bitrate was estimated based on this distribution with a method effectively equivalent to the probabilistic reconstruction approach from Paper 1. However, the approach introduced much less underestimation than the setting of Paper 1, as in the binary protocol used in Paper 3, particular input symbols X_i were independent, which implies equality in Ineq. (12), (compare last row in Box 2).

4.4 Encoding protocol optimization

In Paper 1, protocol optimization was performed through experimental testing of several distributions from three parametric families, and selection the parameter values rendering the highest bitrate. Separation of prior knowledge and gained information in Paper 2 allowed us to estimate bitrate for protocols other than tested experimentally. This let us perform numerical optimization based on results from a single experiment. As the eventual estimates did not differ substantially, the procedure proved that the proposed protocols based on an informed guess were indeed near-optimal. Moreover, it significantly reduced the experimental workload and thus allowed for comparison of multiple cell lines and conditions.

5 Results summary

5.1 Channel capacity of the MAPK/ERK pathway

The main achievement of this publication cycle is showing that the MAPK/ERK pathway can transmit 6-11 bit/h, depending on the cell line and quantification method (Paper 1, Figs. 3&4; Paper 2 Fig. 6F). All estimates were lower bounds; the least biased estimation methods rendered the highest values, which suggests that the true channel capacity of the pathway in normal cells situates around or above 11 bit/h. Such a high bitrate is due to the high chance of evoking a response, short refractory time, and good temporal precision.

We investigated non-tumorigenic epithelial cell lines (MCF-10A, BEAS-2B) and showed that the refractory time of the pathway, understood as the minimum interval after which the response is detected with at least 50% of the asymptotic credibility, is around 8-10 min (Paper 1, Fig. 1G; Paper 2, Fig. 6D). The chance for accurate detection reached saturation for intervals above 15 min, while the temporal precision continued to increase until 20 min (Paper 1, Fig. 1G; Paper 2, Fig. 6D). At saturation, each pulse had over 90% chance of evoking a detectable response; out of this, roughly 85% of pulses were detected timely (up to the 1 min time discretization), while less than 3% erred by more than 1 min (Fig. 1 in this document). Note that the high detectability does not imply full strength of response. On the contrary, we observed that the response amplitude kept rising for intervals well past the 20 min (Paper 2, Fig. 6B), which means that although the system already is functional, it requires significantly more time to fully regenerate.

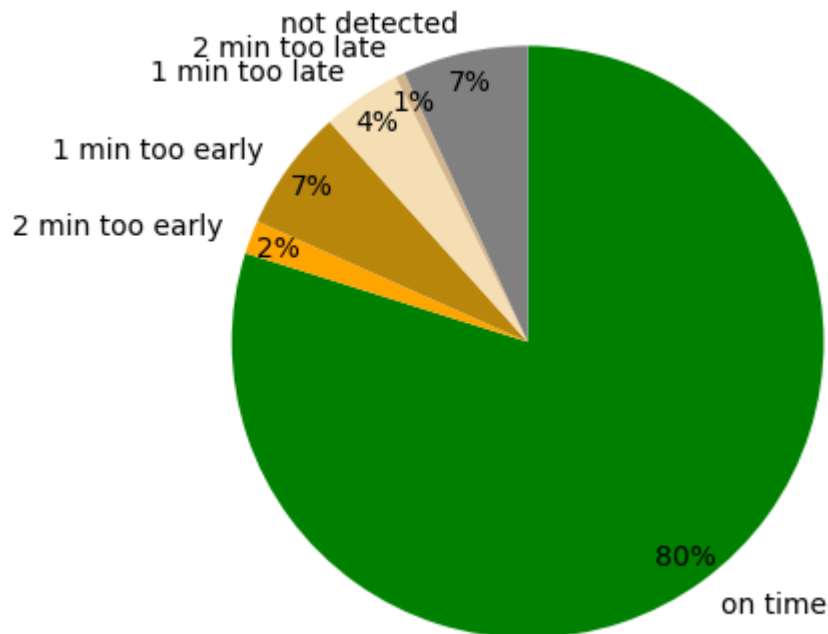


Figure 1. Temporal precision of optoFGFR stimulation reconstruction based on ERK activity in MCF-10A cells. Chance of pulse detection with respect to actual pulse time. Only pulses preceded by an interval of at least 20 min were included. Based on data underlying Fig. 1G in Paper 1.

In most cells, the ERK response to optoFGFR stimulation was reliable and repetitive. However, a fraction of cells (15-70%, highly variable among experimental replicates) notoriously did not respond to stimulation; some but not all of the effect could be attributed to low optoFGFR expression (Paper 2, Fig. S1). The two populations were easily separable (Paper 2, Fig. 3A; Paper 1, S3 Fig.) and the non-responding cells were removed from calculations that lead to results presented above.

In Paper 1, we checked that spontaneous pathway activation is rare and has mild effect on information transmission, while most information loss is due to missed pulses (response not evoked or not clear enough to be detected by the classifier) and imprecise timing (Paper 1, Fig. 2BDF). In protocols forbidding inter-pulse intervals shorter than the refractory time, influence of the last two factors was comparable. This confirmed that the 1-min resolution is close to the temporal precision of the MAPK/ERK pathway as limited by intra- and intercellular noise.

Altogether, the results show that the pathway is able to reliably transmit more than 5 transient activity pulses per hour, which may be used to simultaneously drive several rapid processes like cell movement, phagocytosis or vesicle secretion. The measured channel capacity of 6-11 bit/h markedly exceeds earlier predictions based on a computational model (1.4-1.9 bit/h) (Grabowski et al. 2019), as the refractory time established experimentally was shorter, and temporal resolution higher than assumed in the mentioned work. However, it remains significantly lower than the bitrate through the chemotactic pathway in *E. coli* (0.5-1 bit/min), measured with a linear transmission model according to the Shannon's-Hartley formula (Mattingly et al. 2021).

Our bitrate quantification makes a step towards quantifying information flow in the whole biochemical signaling system, and understanding the cellular decision-making process at the systemic level.

5.2 Influence of EML4-ALK oncogene on bitrate in the MAPK/ERK pathway

In Paper 2, we compared information transmission through the MAPK/ERK pathway in normal cells (BEAS-2B) and non-small-cell lung carcinoma cells (STE-1) expressing a fused EML4-ALK gene. The EML4-ALK fusion protein is known to drive the MAPK/ERK pathway hyperactivity by stimulating RAS.

We measured the bitrate in both cell lines and observed a reduction of the fraction of cells with non-zero channel capacity from ~65% in BEAS-2B cells to below 15% in STE-1 (Paper 2, Fig. 3B). Even in the transmitting subpopulation, the measured bitrate was significantly lower in STE-1 cells (2 bit/h instead of 11 bit/h; Paper 2, Fig. 3C). The effect is a consequence of the nearly full abrogation of response in STE-1 (Paper 2, Fig. 2B).

We demonstrated that the responsiveness in STE-1 cells can be restored by EML4-ALK inhibition, which lowers basal ERK activity (Paper 2, Fig. 2D). Indeed, upon crizotinib (ALK inhibitor) treatment, the fraction of cells able to transmit information became comparable to those of normal cells (Paper 2, Fig. 3B). Despite that, the MAPK/ERK pathway information capacity in treated STE-1 cells was twice lower than in normal (BEAS-2B) cells (Paper 2, Fig. 6D). The immediate reason was mainly the lower probability that a stimulus induced a detectable response, especially after intervals close to the refractory time. We showed that the underlying cause was probably the lower signal-to-noise

ratio (Paper 2, Fig. 3D), which might have originated from the pathway impairment despite or due to crizotinib treatment, but also from inevitable technical differences in the experimental procedure.

5.3 Bitrate in calcium signaling

As a side result of Paper 2, we provided an estimate (lower bound) for channel capacity of the optoFGFR-PI3K-IP₃-Ca²⁺-calcineurin pathway, which could be examined using the same stimulator/reporter pair. Compared to the MAPK/ERK pathway, the calcineurin pathway proved to have lower latency of around 2 min and higher bitrate of at least 14 bit/h. In natural conditions, the two pathways converge at the level of a transcription factor ELK-1; the information from both pathways could potentially be integrated at that point to exert the desired cell behavior.

5.4 Long-range information transmission in a cell monolayer

In Paper 3, we analyzed a model of juxtacrine-mediated activity propagation. We showed that – for the parameter set inspired by mechanochemical ERK waves – fronts of cellular activity can propagate and transmit information at rates of the order of 0.5 bit/h over the distance of hundreds of cells, provided that the reactor has proper width (Paper 3, Fig. 4).

This ability is sensitive to changes in model parameters (Paper 3, Fig. 6D) and reactor geometry (Paper 3, Fig. 4CE). We identified two crucial parameters: the probability that an active cell stimulates a neighbor before proceeding to the refractory state, and the ratio between the refractory time and the time from cell's stimulation to its deactivation (Paper 3, Fig. 5). The first parameter determines the probability that a front spontaneously disappears, which is limiting for information transmission in extremely narrow geometries (<6 cells wide), but plays a marginal role in wider channels. In contrast, the second parameter affects the propensity of new front spawning, described in detail below, which is crucial in broader geometries. Long refractory time compared to activation delay and period of activity promotes stable propagation. This highlights the pivotal goal in minimizing activation delay, and the ambiguous role of refractory time, which limits the maximum front frequency, but facilitates their undisrupted propagation.

5.5 Front spawning is a major cause of information transmission disruption

While the original aim of the project was to measure, how much information can be transmitted over long distances by fronts of cell activity, in the final form of the paper we put much attention to characterization of disruptive stochastic phenomena occurring in this excitable medium.

We identified three major causes disruption of front propagation (Paper 3, Fig. 2):

- a) stochastically varying speed of front propagation,
- b) spontaneous disappearance of fronts when all cells simultaneously lose activity,
- c) creation (spawning) of new fronts behind a propagating front.

While the first two phenomena were expected, the significance of the last one came to us as a surprise. In our model, front spawning occurs when a cell remains active long enough so that a neighboring cell recovers from the refractory state and can be reactivated. Such an event can lead to creation of a front or fronts propagating in the same and/or another direction as the original front.

It may also turn into a long-lasting ‘spawning site’ that emits new fronts in both directions at regular intervals. The ‘spawning sites’ are similar to structures observed in a cellular automaton by (Szakály et al. 2007), Fig. 7. It depends on the model parameters and reactor width whether front extinction or front spawning is more probable. We observed that in narrow reactors, a single backward front is typically spawned, while in broad ones, spawning sites are usually formed. In the intermediate regime, one or more pairs of backward and forward front are created.

The frequency of spawning events increases when two fronts propagate in quick succession. Consequently, the newly created fronts have an increased chance of initiating a new spawning event.

The spawned fronts influence information transmission encoded in the sequence of fronts. A backward front will eventually collide with another front and as a result the two fronts will almost certainly annihilate (disappear). The net outcome of the spawning event would be the loss of information carried by the annihilated front, which is functionally equivalent to the front’s spontaneous disappearance. Fronts spawned forward would render additional arrivals at the reactor end, generating misleading information. This can however – at least theoretically – be eliminated, as the fronts spawned forward propagate with a standard delay after the original front, and can thus be distinguished from information-carrying fronts.

While spawned fronts cause moderate disruptions, occurrence of a spawning site is catastrophic to the system, as a spawning site blocks any information transmission as long as it exists. Moreover, fronts it generates may create new spawning sites, causing a chain reaction and thus significantly prolonging the system’s blockage. The frequency of spawning site occurrence is proportional to the time the fronts spend in the reactor, which scales with the reactor length, and the propensity of spawning site creation, which is proportional to the reactor width. Consequently, there exists a critical volume (surface) of the reactor, above which the chain reaction is sustained and thus no long-term information transmission is possible.

5.6 Optimal reactor width is several cells wide

The propensity of spontaneous front disappearance decreases (exponentially) with the reactor width, as all cells on the front must lose their activity simultaneously. On the other hand, the chance for a spawning event increases (linearly) with the reactor width, as each cell on the front might become a source. As both processes impair information transmission, there must be an optimal width. We found that for the parameter set inspired by ERK waves in epithelium, the optimal reactor is only 6 cells wide – in such a reactor, coherent information transmission is possible over a distance of hundreds of cells. The optimal width scales roughly linearly with model parameters, such as activity time or refractory period, therefore will remain low for a broad range of biologically relevant parameters.

6 Results in the context of the research hypotheses

In Papers 1 and 2 we showed that the MAPK/ERK pathway is able to reliably transmit 5-7 pulses per hour with 1 min temporal resolution, which is reflected in the high channel capacity of 11 bit/h, markedly exceeding earlier model-based estimates (Grabowski et al. 2019). Mechanochemical waves of ERK activity and cell contraction, driving collective cell motion, occur with a period of around 45 min (Hino et al. 2020). The same temporal scale is associated with various transcription-based processes, due to the minimum time required for protein synthesis. Our results show that during that time, 4-5 pulses can be evoked, carrying ~8 bits of information. This proves the pathway's potential ability to simultaneously handle multiple such processes.

We demonstrated that the pathway's ability to transmit information is almost completely abolished in cells expressing the EML4-ALK oncogene, which induces constitutive activation of the pathway. This is connected with the lack of detectable ERK response to optoFGFR stimulation, rather than temporal imprecision or spontaneous pulse occurrence. Inhibition of the oncogene with crizotinib at least partially restored the channel capacity. In crizotinib-treated cells, the temporal resolution was measurably decreased compared to normal cells, although this decrease played an inferior role in bitrate reduction (Figure 2 in this document), which was mainly due the lower amplitude, implying worse signal-to-noise ratio (Paper 2, Fig. 3D).

Using a numerical model, we showed that fronts of cell activity can transfer information over distances of hundreds of cells. However, such transfer is possible only in systems several cells wide. Biological examples of such systems include capillary or lymphatic capillary walls. According to our model, in broader systems, like skin or mucosa, the maximum range is significantly reduced, as prolonged front propagation results in chaotic front spawning patterns. Such patterns were observed *in vitro* by (Hino et al. 2020). Stability of front propagation is governed by the refractory time. Its prolongation could extend the range of information transmission, but would decrease bitrate in shorter systems. Such behavior has been observed in ERK waves in fish scale regeneration, in which case the observed time interval between fronts was of the order of a day rather than an hour (de Simone et al. 2021; Hayden et al. 2021).

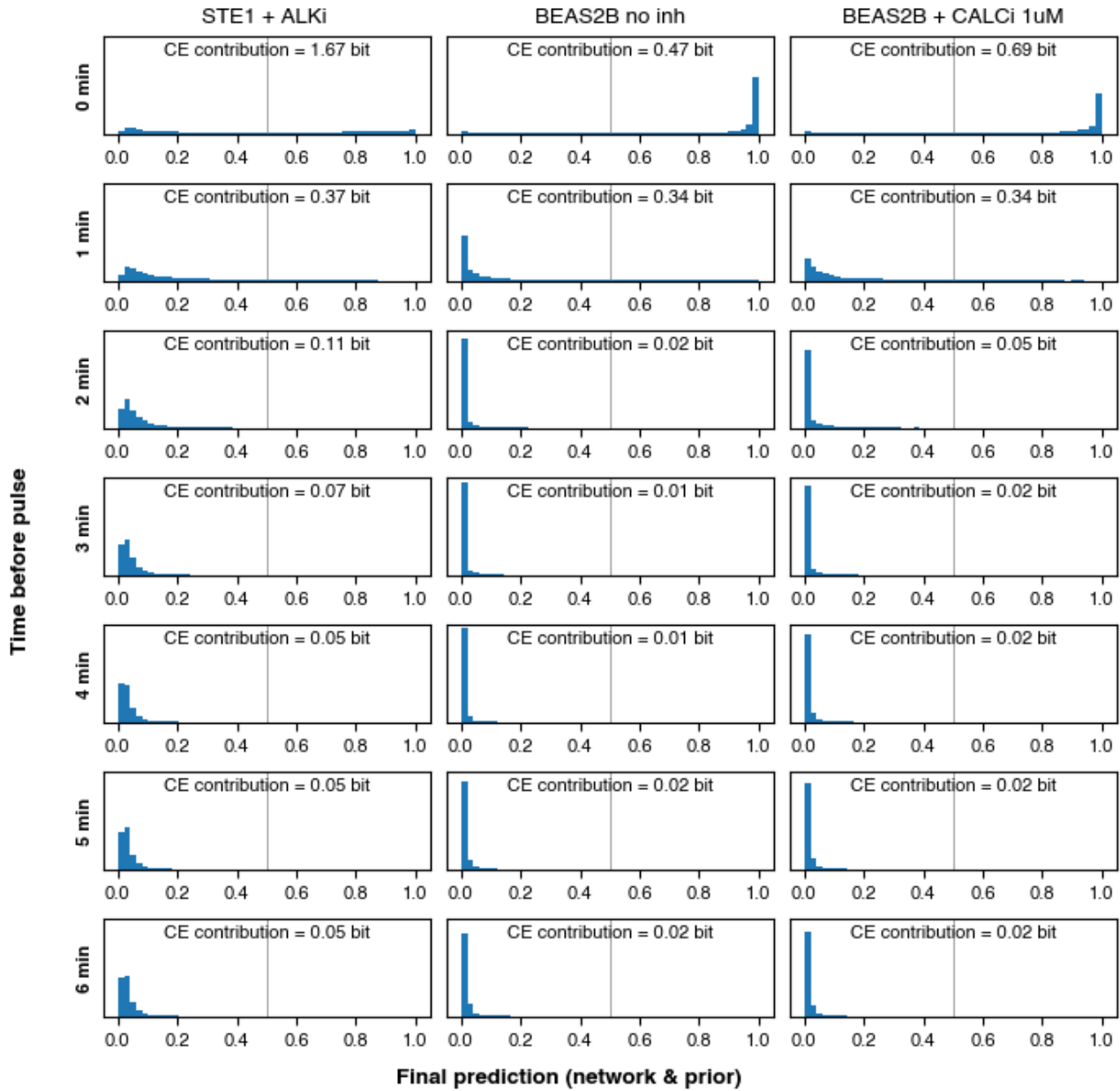


Figure 2. Impact of temporal resolution on information transmission rate through the MAPK/EKR pathway Histograms show the probability of pulse occurrence, as predicted by the classifier based on single-cell ERK KTR trajectories, at timepoints preceding the actual pulse by the time specified for each row. Columns represent three different conditions (cell line, inhibitor). Contribution to the cross-entropy between the prediction and the actual pulse occurrence (i.e., to the information loss) are reported in each panel.

Note that the cross-entropy contribution at pulse (first row) is an approximation of the information lost due to missed pulses (1.7 bit/pulse, i.e. 5.7 bit/h, for ALKi-treated STE-1 cells), while the remaining contributions reflect the inability to exclude pulse presence at other time points (asymptotically, around 0.05 bit/timepoint, i.e., 3 bit/h, for ALKi-treated STE-1 cells). The total excess information loss at time points 1-3 min before actual pulse (around 0.40 bit/pulse, i.e., 1.4 bit/h for treated STE-1) is a result of temporal imprecision of the pathway. The last factor is least variable between cell lines.

Data from Paper 2. Only timepoints from intervals at least 10 min long included. Data preprocessing as in Paper 2. Only cells with proper amount of optoFGFR from the transmitting subpopulation included.

7 Conclusion and perspectives

In papers constituting this thesis, the rate of pulse-encoded information transmission through a biochemical signaling pathway was estimated for the first time. Obtained numerical values are relatively high, compared to earlier predictions, which shows that pulse timing can be a potent carrier of information in cellular signaling.

In contrast, numerical simulations indicate that long range information transmission by juxtacrine-mediated activity fronts in a cell monolayer is a challenging task in populations more than several cells wide. In such broad systems, undisrupted long-range propagation of fronts was shown to be conditioned on relatively long refractory time, which in turn limits bitrate. The results qualitatively agree with observed front propagation patterns in migrating cells (Hino et al. 2020), but a dedicated experimental study would certainly strengthen the conclusions and can be suggested for further research.

While the presented methods are generic, the biological results are limited to a single signaling pathway – the MAPK/ERK pathway. This does not allow comparison across various systems. Moreover, it is clear that processing of information by the cell must reduce the high-throughput perceptive information to a relatively low-rate sequence of behavioral decisions. Quantifying bitrate at various stages of processing could help identifying key steps in decision-making. Therefore, measurement of bitrate transmission in other signaling pathways and particular components thereof would open a way to creating a quantitative understanding of information flow in the cell.

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Papers

RESEARCH ARTICLE

The MAPK/ERK channel capacity exceeds 6 bit/hour

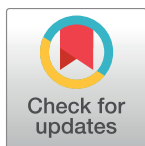
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Data Availability Statement: A complete set of live-cell imaging data is available at DOI: [10.5281/zenodo.7808385](https://doi.org/10.5281/zenodo.7808385) Source code used to generate main figures is available at URL: <https://github.com/pawelnaiecz/pulsatile-information>.

Abstract

Living cells utilize signaling pathways to sense, transduce, and process information. As the extracellular stimulation often has rich temporal characteristics which may govern dynamic cellular responses, it is important to quantify the rate of information flow through the signaling pathways. In this study, we used an epithelial cell line expressing a light-activatable FGF receptor and an ERK activity reporter to assess the ability of the MAPK/ERK pathway to transduce signal encoded in a sequence of pulses. By stimulating the cells with random light pulse trains, we demonstrated that the MAPK/ERK channel capacity is at least 6 bits per hour. The input reconstruction algorithm detects the light pulses with 1-min accuracy 5 min after their occurrence. The high information transmission rate may enable the pathway to coordinate multiple processes including cell movement and respond to rapidly varying stimuli such as chemoattracting gradients created by other cells.

Author summary

To respond to external stimulation, living cells need to transfer information from membrane receptors to downstream proteins that regulate physiological outcomes. In this study we used cells expressing a light-activatable receptor that transmits signals to MAPK/ERK transduction pathway, which regulates numerous physiological processes including proliferation, differentiation and cell motility. By stimulating these cells with various sequences of short light pulses we found that ERK, the last component of the considered signal transduction pathway, may receive at least 6 bits per hour. This bitrate allows 6 binary decisions per hour, enabling rapid responses to varied extracellular stimuli.

Introduction

Living cells communicate with each other and constantly monitor their extracellular milieu. Upon receiving a stimulus, they have to recognize its identity and act accordingly to operate in a coordinated manner and properly adapt to the changing environment. At the molecular

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level, recognition of a specific signal as well as its reliable transmission and appropriate interpretation involve diverse intracellular processes that are notoriously stochastic [1]. At the single-cell level, major intracellular signaling pathways respond to an external stimulus in a crude all-or-nothing manner [2–5]. The inability of individual cells to resolve the level of stimulation is reflected in the amount of information about the ligand concentration the signaling pathways can transmit, which has been estimated to only slightly exceed 1 bit [6–9].

However, when cells are subjected to temporally varying stimulation by morphogens, hormones, or cytokines, it is of interest to quantify the maximum rate at which information can be transferred through a biochemical signaling pathway. A similar question has been asked before in the fields of neuroscience and bacteria or amoebae chemotaxis. Neurons utilize precise timing of spikes to evoke proper response [10–12]. The information transmission rate (or simply bitrate) through a single neuron may reach tens of bits per second [13,14]. For bacteria navigating in a chemical gradient, the drift velocity scales linearly with the gradient, while the bitrate was found to be proportional to the square of the gradient [15]. When concentration varies on the length scales of millimeters to centimeters, the chemotactic system of *E. coli* transfers about 0.01 bit per second, which allows bacteria to climb up the gradient with a few percent of its swimming speed. This suggests that at low gradients, drift velocity is information limited [15]. Without explicitly quantifying the information flow, Meier *et al.* [16] showed that *Dictyostelium discoideum* amoebae may synchronously readapt their migration direction to switching chemotactic gradient oscillating at rates up to 0.02 Hz. This suggests that the rate of information transmission to the navigation system is up to 1 bit per minute, and the limit is achieved if all cell decisions are correct. The maximum bitrate depends on biophysics of a specific information channel. In the case of neurons, where the bitrates are highest, information is transmitted with the help of fast electro-chemical signals; in the case of *E. coli* and MAPK, information is transmitted by means of posttranslational proteins modifications, thus expected bitrates are lower. The bitrate observed in the *E. coli* chemotactic system can be higher than that expected for the MAPK channel, because bacteria are much smaller than a eukaryotic cell. Some other signaling pathways such, as that of NF- κ B or p53, require protein synthesis and degradation, which are slower than post-translational modifications, and so for these pathways even lower bitrates are expected.

Gagliardi *et al.* [17] demonstrated that the ERK pulses triggered locally by growth factors shed by apoptotic cells coordinate the maintenance of epithelial integrity. *In vivo* studies showed pulsatile ERK dynamics during wound healing in mice, wherein the wound edge initiates recurrent, quasiperiodic mechanochemical traveling fronts of ERK activity [18]. Such waves were also observed during mammalian epidermis development [19] and during scale regeneration in zebrafish [20]. Pulsatile ERK dynamics has been observed also for tonic EGF stimulation, for example Albeck *et al.* [21] and Aoki *et al.* [22] have shown that the MAPK pathway converts various types of stimulation into ERK pulses, suggesting their role in generating physiological outcomes.

In this study, we assessed the capability of the MAPK/ERK pathway to transduce information encoded in a sequence of stimulatory pulses, and estimated the lower bound for the rate of information transmission through this pathway. In light of the mentioned findings, we focused solely on pulsatile stimulation protocols. As we conjecture that protocols ensuring highest information transfer are used for cell signaling, we searched for a protocol that would maximize the information transmission rate, and thus estimated the MAPK/ERK channel capacity. We considered three different families of pulsatile input protocols: (i) *binary encoding*, resembling digital communication with zeros and ones occurring in well-defined, temporally equidistant time slots, is plausibly the simplest pulsatile stimulation protocol; (ii) *interval encoding*, with exponentially-distributed waiting times in discrete time domain (so, effectively,

geometrically-distributed waiting times), is statistically maximally random (entropic); this protocol is also natural from the biological perspective as it implies constant propensity of receiving stimulation pulses in time; (iii) *interval encoding with an additional gap* after each pulse is an attempt to tailor a maximally entropic stimulation to cells characterized by an inextricable refractory (resting) period. We used an epithelial cell line with an optogenetically modified fibroblast growth factor receptor (optoFGFR) [23,24] which allowed us to stimulate the pathway with short light pulses activating ERK with the similar kinetics as EGF or FGF [25]. The cell line is stably transfected with a reporter, enabling fine temporal monitoring of ERK activity. By stimulating cells with random pulse trains, generated with probabilistic algorithms according to protocols from the three families, and applying classifier-based algorithm to reconstruct the stimulatory sequence, we demonstrated that the maximum information transmission rate through the pathway exceeds 6 bits per hour. A classifier-free approach gives an even higher estimate of about 8.5 bits per hour.

Results

Information encoding and information transmission rate

The maximum amount of information that can be transmitted through a communication channel per unit time using the best possible input protocol is called the channel capacity C :

$$C = \max_{\text{protocols}} \lim_{\Delta t \rightarrow \infty} \frac{I(S, R)}{\Delta t} \quad (1)$$

where $I(S, R)$ is the mutual information between the input (signal) S and the output (response) R in an experiment of duration Δt [26]. In this work, we computed $I(S, R)$ as the difference:

$$I(S, R) = H(S) - H(S|R) \quad (2)$$

where $H(S)$ is the input entropy (the amount of information sent), which in our experimental design can be computed directly from the assumed probabilistic distribution of the pathway-stimulating input sequence; and $H(S|R)$ is the conditional entropy (the amount of information lost), which captures the uncertainty introduced by the channel (here: the signaling pathway). The value $H(S|R)$ depends both on the channel and the signal and as such is estimated based on experimental data.

There are multiple ways to encode information in a train of pulses. When the magnitudes of all stimulation pulses are equal, the input can be perceived as a sequence of 0s (no pulse) and 1s (pulse). The input information (or entropy) rate increases with the frequency of pulses, reaching the maximum for binary sequences that have equal probabilities of 0 and 1. However, shorter intervals between pulses imply higher information loss due to imperfect transmission. One should thus expect that there exists an optimum, for which the bitrate is maximal.

We applied three types of pulsatile stimulation protocols to estimate the lower bound for the channel capacity of the MAPK/ERK pathway (see Fig 1A and Materials and methods for details). In what we call the *binary encoding protocol*, information is encoded in a sequence of 0s and 1s sent at regular time intervals τ_{clock} with equal probabilities. In our experiments, we stimulated cells with light pulses according to a 0/1 sequence of length 19: 1011010011110000101 and its logic negation (both of them containing each of the 16 possible subsequences of length 4 exactly once, which allowed us to collect good statistics in a single, relatively short experiment). In the *interval encoding protocol*, information is encoded in the lengths of intervals between subsequent input light pulses. To maximize the input entropy (which, in light of Eq (2), bounds the transmitted information from above), the intervals were drawn from geometric distribution. In the *interval encoding protocol with a minimal gap*, the

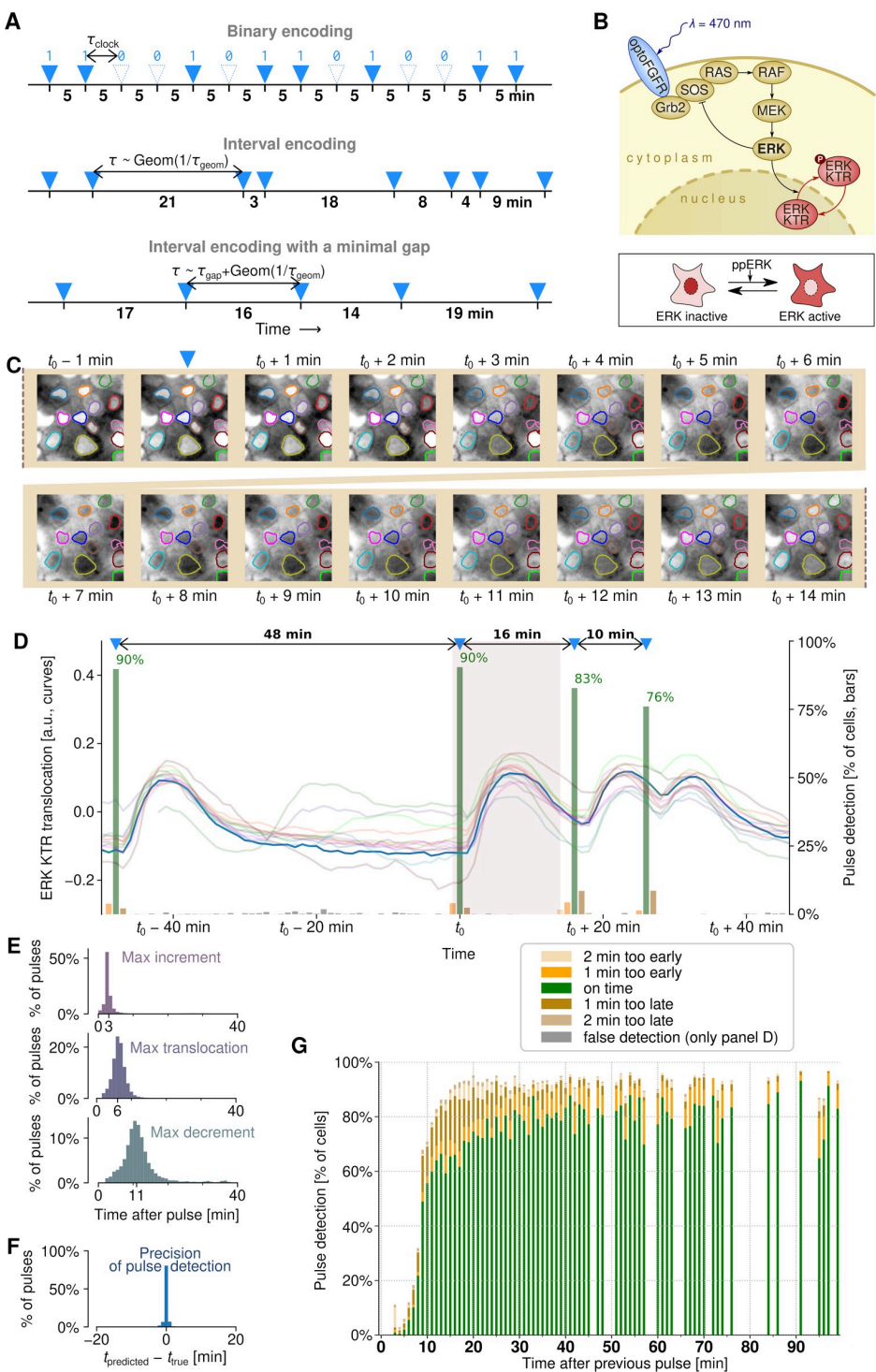


Fig 1. Decoding pulsatile signals relayed through the MAPK/ERK pathway. (A) Input: three considered protocols of encoding information in a pulse train. In binary encoding, a pulse is either present or not in each of the temporally equidistant slots. In interval encoding, information is carried by the lengths of time intervals between subsequent pulses. The intervals are drawn from a geometric distribution (with one-minute resolution). In interval encoding with a minimal gap, the intervals are drawn from a geometric distribution and a fixed “gap” is added. Small numbers in bold typeface denote intervals between pulse slots (in binary encoding) and inter-pulse intervals (in both interval encodings). (B) Diagram of the MAPK/ERK pathway within the engineered MCF-10A cells. Blue light activates

optoFGFR, triggering a kinase cascade, which culminates in ERK activation. The fluorescent (mRuby2) ERK KTR, which in non-stimulated cells is mostly localized to the nucleus, upon phosphorylation by active nuclear ERK (ppERK) is exported to the cytoplasm. (A drop of) the mean nuclear fluorescence of ERK KTR is used as a proxy of its translocation and ERK activity. (C) Output: ERK KTR translocation in response to activation of optoFGFR by a light pulse at t_0 . Nuclear contours of 13 cells are marked with different colors. For the sake of presentation, microscopic images are normalized such that the original 10%–90% quantiles of pixel intensity span over the whole grayscale range, black to white. (D) Input reconstruction: time track of ERK KTR translocation in response to a sequence of 4 light pulses in the 13 cells marked in panel C. The shaded interval is the interval in which the snapshots shown in panel C were acquired; the trajectories correspond to respective color-coded nuclear outlines shown in panel C. Green bars show the proportion of cells (estimated based on 400 single cell trajectories) in which a pulse was detected by the trained classifier. (E) Histograms of three basic temporal features characterizing the ERK KTR translocation profile: time to the largest translocation increment, the peak of translocation, and the largest translocation decrement (all with respect to the time of a light pulse that elicited the characterized response). In a typical cell, the translocation has the steepest slope between 2 and 3 min after the light pulse, reaches the maximum at 6 min, and rebounds at the highest rate between 10 and 11 min after the light pulse. Data from all 6 experiments with the interval encoding protocol. (F) Accuracy of the light pulse detection. Most of the pulses are detected exactly with one-minute resolution. Data as in panel E, classifier trained on other cells from the same experiment. (G) Proportion of cells in which a pulse was detected as a function of the interval after the previous pulse. Data as in panel F.

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time intervals were also drawn from geometric distribution (with the average inter-pulse interval τ_{geom}), and then τ_{gap} was added to the drawn value, thus the expected value of the inter-pulse interval was $\tau_{\text{geom}} + \tau_{\text{gap}}$. For each τ_{gap} , we chose τ_{geom} that maximizes the input entropy (see [Materials and methods](#) for details). Introduction of τ_{gap} reduces the input entropy rate compared to the interval encoding protocols, but increases the percentage of cells evoking a timely response to a light pulse, which facilitates accurate information transmission.

Detection of pulses

To quantify the bitrate through the MAPK/ERK pathway, we used the human mammary epithelial cell line MCF-10A with both an optogenetically modified fibroblast growth factor receptor (optoFGFR) [17,25], which can be activated with blue light (470 nm), and a fluorescent ERK kinase translocation reporter (ERK KTR), which translocates from the nucleus to the cytoplasm when phosphorylated by ERK (Fig 1B). The cells were stimulated with short (100 ms, see [Materials and methods](#)) blue light pulses according to the three types of encoding protocols (Fig 1A). ERK KTR translocation was measured at one-minute resolution (Fig 1C and 1D; see [Materials and methods](#) and [S1 Fig](#) for details of the workflow). We observed that the signal was transduced from optoFGFR to ERK KTR within single minutes, consistently with Ref. [25], with the steepest translocation increment (drop of the nuclear ERK KTR fluorescence) observed between 2 and 3 min, maximum translocation at 6 min, and steepest translocation decrement around 11 min after the light pulse (Fig 1E).

We then employed a classifier based on the k -nearest neighbors (k NN) algorithm (see [Materials and methods](#) for details) to reconstruct the sequence of stimulatory light pulses using the quantified single-cell trajectories of nuclear ERK KTR (Fig 1D). The algorithm was designed to predict the timing of light pulses using a short (overall, 8 minute-long) window sliding over the ERK KTR translocation trajectory. The method was typically able to predict the pulse timing with one-minute resolution (Fig 1F), markedly better than any individual pulse feature (cf. Fig 1E and 1F). The percentage of detected pulses increases sharply with the time interval between the pulse to be detected and the previous pulse, reaching about 70% for the interval of 10 min and nearly 90% for the interval of 15 min (Fig 1G). Missed pulses, pulses detected as occurring earlier or later (proportion of which is shown in Fig 1G), and false detections are responsible for the information loss.

Bitrate estimation

We computed the information loss along the pathway as the entropy of the input conditioned on its (classifier-based) reconstruction from single-cell ERK KTR trajectories, $H(S|R)$. Next, by subtracting the information loss from the input entropy $H(S)$, we quantified the transmitted information $I(S|R)$, and eventually, by dividing the result by experiment duration, we estimated the bitrate. It is important to stress that our method of pulse detection is based only on an 8-min rolling window, hence we do not use prior knowledge about the minimal gap between pulses.

In Fig 2A we show 10 example single-cell ERK KTR translocation trajectories of cells responding to the first sequence used in the binary encoding protocol with the time span between subsequent digits (clock period) $\tau_{\text{clock}} = 10$ min. The percentage of cells identified as responding to light pulses varied from around 80% to nearly 100%. As expected, the detectability is higher when the time span from the preceding pulse is 20 min or more (more than 95% pulses are detected). A tiny percentage of cells, $\leq 1\%$, was incorrectly identified as responding at time points without a light pulse.

For the binary protocol we performed experiments for 7 different clock periods ranging from 3 to 30 min (Fig 2B). The input information rate, $1 \text{ bit}/\tau_{\text{clock}}$, decreases with increasing τ_{clock} but, as expected, for longer τ_{clock} the fraction of information lost due to missed pulses or false detections is lower. For the shortest τ_{clock} of 3 min, the input information rate reached 20 bit/h, but due to severe information loss the transmitted information rate was lower than for $\tau_{\text{clock}} = 5$ min (Fig 2B). It should be noted that in the case of the binary encoding protocol during input reconstruction we utilize the *a priori* knowledge of the time points in which the light pulses could have occurred (“pulse slots”). Thus, based on the ERK KTR translocation trajectory we have to decide only whether the light pulses occurred in these time points or not. This makes the reconstruction easier than in the interval encoding protocols, however binary encoding appears rather artificial in the biological context.

In Fig 2C we show a representative part of ERK KTR translocation trajectories in 10 cells responding to stimulation with light according to the interval encoding protocol with $\tau_{\text{geom}} = 32$ min. For all but one input pulse the percentage of cells identified as responding timely exceeds 75% and is generally higher for pulses occurring after a longer time span from the previous pulse. For inter-pulse intervals below 10 min, the detectability decreases rapidly (for example, the pulse at minute 744 that occurred 8 min after its predecessor was detected timely by only 25% of cells). For each pulse there is also a fraction of detections that indicate an input pulse 1 min before or after the true pulse.

We performed 6 experiments according to the interval encoding protocol with τ_{geom} ranging from 22 to 55 min (Fig 2D). The highest bitrate of 6.3 bit/h was found for $\tau_{\text{geom}} = 36$ min, while for $\tau_{\text{geom}} = 32$ and 42 min the bitrate was found equal 5.3 and 5.0 bit/h, respectively. The shortest (22 min) and longest (55 min) average intervals resulted in, respectively, the highest and the lowest information loss. This causes that for these two suboptimal protocols, having respectively high (15 bit/h) and low (7.7 bit/h) input information rate, the bitrate is similar, between 4 and 5 bit/h. The information is lost due to the imprecise detections (that carry incomplete information about the timing of pulses), missed pulses and false detections (Fig 2D). The estimation of the information loss due to these three types of signal reconstruction errors is outlined in S2 Fig, panels C–E, and described in Materials and methods.

In Fig 2E, we show 10 example trajectories for the interval encoding protocol with $\tau_{\text{geom}} = 10$ min and a gap of $\tau_{\text{gap}} = 20$ min (giving together the mean inter-pulse interval equal 30 min). The ERK KTR translocation pulses are well distinguishable and thus in around 90% of single-cell trajectories the stimulation pulses were reconstructed with one-minute resolution.

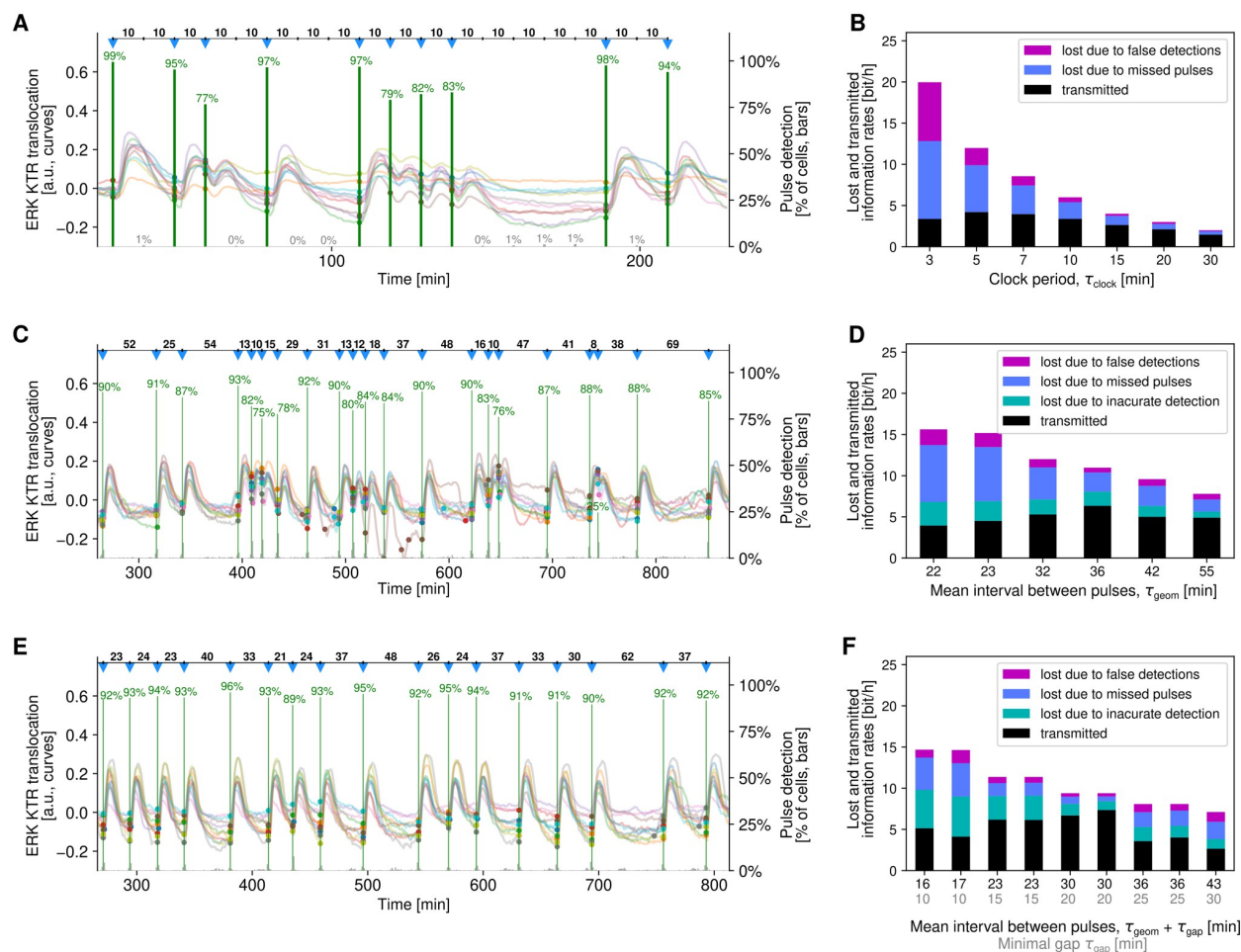


Fig 2. Pulse detection and bitrate estimation in three protocols. (A) Binary encoding: 10 representative trajectories from an experiment with slots every $\tau_{\text{clock}} = 10$ min. Stimulation pulses are marked with blue down-pointing triangles. Small filled circles indicate light pulses detected based on the corresponding trajectories. Percentage-labeled green bars in each pulse slot show the fraction of cells in which a pulse was correctly detected. Fractions of false positive detections are shown as percentage-labeled gray bars. (B) Binary encoding: bitrate and sources of bitrate loss in experiments with various clock periods. In each column, total height of the stacked bars is equal to the input entropy rate. The number of analyzed cell trajectories was 3200 for clock periods τ_{clock} ranging from 3 to 15 min and 1200 for $\tau_{\text{clock}} = 20$ and 30 min. Each analyzed cell trajectory contained 19 pulse slots. (C) Interval encoding: 10 representative trajectories from the experiment with the mean interval $\tau_{\text{geom}} = 36$ min. Stimulation pulses are marked with blue down-pointing triangles. Colored dots indicate light pulses detected based on the corresponding trajectories. Green bars show the fraction of cells in which a pulse was detected in a particular minute. (D) Interval encoding: bitrate and sources of bitrate loss in experiments with various mean intervals. In each column, total height of the stacked bars is equal to the input entropy rate. The number of analyzed cell trajectories was 400 in each of 6 experiments. The mean number of light pulses in analyzed trajectories was in the range 24–60 depending on the mean interval (all experiments lasted 27–33 h). (E) Interval encoding with a minimal gap: 10 representative trajectories from the experiment with a minimal gap of $\tau_{\text{gap}} = 20$ min and the inverse of the optimized geometric distribution parameter $\tau_{\text{geom}} = 10$ min. Graphical convention as in panel C. (F) Interval encoding with a minimal gap: bitrate and loss sources in experiments with various minimal gaps and mean intervals. In each column, total height of the stacked bars is equal to the input entropy rate. The number of analyzed cell trajectories was 400 in each of 9 experiments. The mean number of light pulses in analyzed trajectories was in the range 19–96 depending on the mean interval (all experiments last 27–33 h).

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For this protocol the bitrate was found to be the highest, equal 7.0 bit/h based on two experimental replicates (Fig 2F). The somewhat lower bitrate of 6.2 bit/h was found for the protocol with $\tau_{\text{geom}} = 8$ min and $\tau_{\text{gap}} = 15$ min (average of two replicates). As for the previous protocol, information is lost due to the three types of errors.

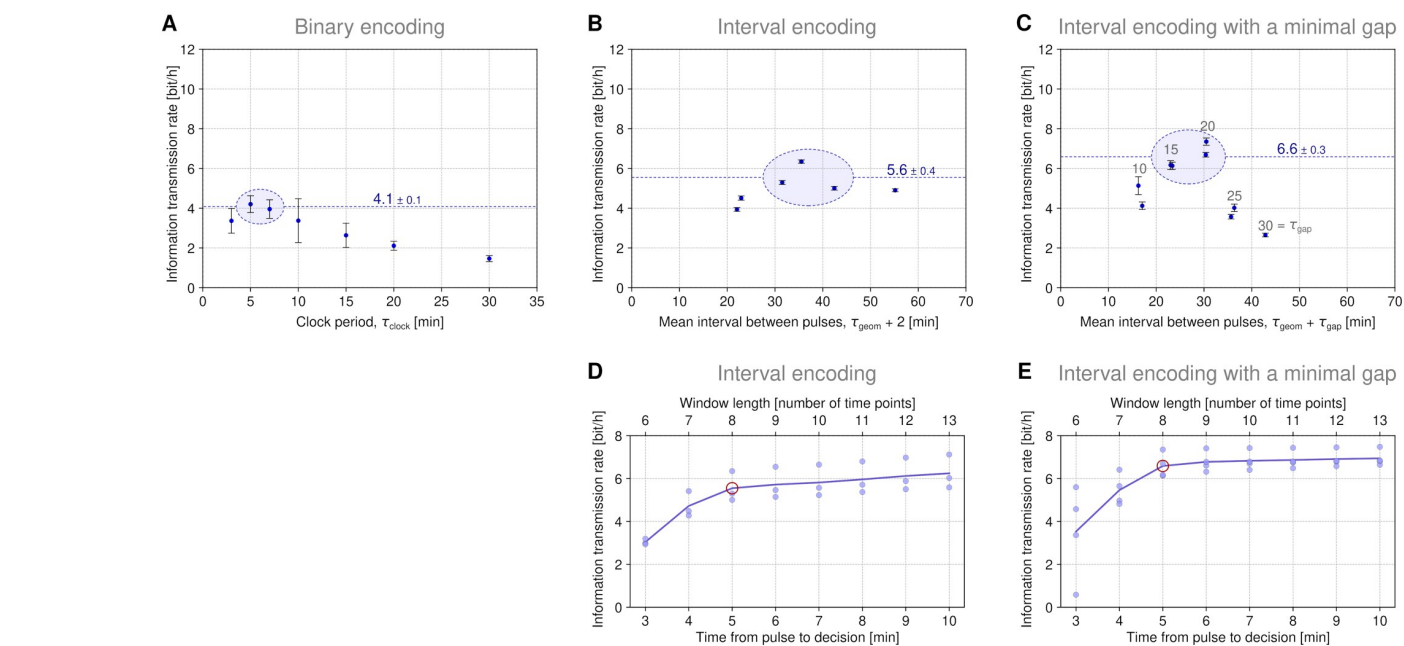


Fig 3. Channel capacity estimation–reconstruction-based approach. (A)–(C) Information transmission rate for the three protocol families. In A, each data point is a mean of 8–10 experiments, and standard deviation of these replicates is shown. In B and C, where each point corresponds to a single replicate, the standard deviation of the estimates obtained after 10 random train–test splits of the 400 cell trajectories is shown. The maximum bitrate for a given protocol is estimated (from below) as the mean $\pm$ standard error of the mean of 2–4 encircled points and marked with a dashed line. (D)–(E) Lower bound of maximum bitrate as a function of time from pulse to its detection for two interval encoding protocols. Points correspond to experiments encircled in panels B and C; line shows the average over these experiments. Pulse detection is based on the ERK KTR trajectory window that begins 2 min before a pulse slot and ends at the time after the pulse slot indicated on the horizontal axis (classification is always based on three overlapping slices of their length adjusted to the length of the window). The 8 min-long window (red circle) was used for pulse detection throughout the paper.

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Latency and accuracy of signal detection

In Fig 3A–3C we gathered all the experiments to estimate the maximum bitrate that can be achieved across all 3 protocols. From 6 to 9 experiments performed for each protocol we selected 2–4 experiments for which we obtained the highest bitrate, and estimated the lower bound of the maximum bitrate as the average over these selected experiments. As a result, we obtained the following bounds (mean $\pm$ standard error of the mean):

- 4.1 ± 0.1 bit/h for the binary encoding protocol with the optimal clock period (τ_{clock}) in the range of 5–7 min,
- 5.6 ± 0.4 bit/h for the interval encoding protocol with the optimal average interval (τ_{geom}) in the range of 32–42 min, and
- 6.6 ± 0.3 bit/h for the interval encoding protocol with a minimal gap, with the optimal gap interval (τ_{gap}) in the range of 15–20 min and the (inverse of the) corresponding geometric distribution parameter (τ_{geom}) in the range of 8–10 min.

Altogether, the above results show that the channel capacity of the MAPK/ERK pathway exceeds 6 bit/h and suggest that the highest bitrate can be achieved for the interval encoding protocol with a gap.

We should mention that the bitrate estimations were obtained after rejecting the fraction $f = 20\%$ of ERK KTR trajectories that exhibit the lowest variability, that is, those with the smallest average square change of the nuclear intensity between subsequent snapshots. As we can see in [S3 Fig](#), panel A, the maximum bitrate estimated for each of three protocols is an increasing function of f for f in the range from 0 to 50%. In the case of the binary encoding, the maximum bitrate increases nearly linearly with f , which results from the fact that for short clock periods lower variability directly corresponds to lower chance of responding to a light pulse. However, for the interval encoding protocol the most significant increase is observed in the range 0–20%, which motivated us to remove 20% trajectories. At least some of these trajectories come from cells with optoFGFR or ERK KTR not functioning properly.

In [S3 Fig](#), panels B–D, we show the histograms of bitrate measured for single cells in all experiments for the three encoding protocols. The orange area in the histograms corresponds to the removed 20% fraction of cells. We can notice that the histograms for the interval encoding protocol with a minimal gap are bimodal, but by removing the 20% of cells we may eradicate the lower mode, which additionally justifies the choice of the particular value of f (for consistency we removed the same fraction of trajectories from experiments for the other two protocols).

All bitrate estimations are based on the ERK KTR translocation trajectory analyzed in an 8 min-wide rolling window that ends $t_D = 5$ min after the hypothetical pulse to be detected (t_D stands for ‘time to decision’). This means that we account for information that is available for each cell 5 min after the pulse that the cell is expected to recognize. In [Fig 3D](#) we give the maximal bitrate estimates (for the interval encoding protocol with a minimal gap jointly for the experiments with the highest bitrate, encircled in [Fig 3A–3C](#)) as a function of t_D . We found that increasing t_D beyond 5 min only marginally increases the bitrate, while decreasing it below 3 min dramatically decreases the bitrate (which possibly reflects the fact that the maximum increment is usually observed between 2 and 3 min after the pulse).

Reconstruction-free bitrate estimation

An advantage of the reconstruction-based approach is that it enables discerning particular sources of classification errors (as shown in [Fig 2B](#), [2D](#) and [2F](#)) and provides insight into information available to a responding cell. On the other hand, any classifier loses some information about the responding cell and thus provides a lower bound on the estimate of the cell capability of transmitting information. Therefore (prompted by an anonymous Reviewer), we performed a direct (reconstruction-free) estimation of the information transmission rate for both families of interval encoding protocols. We used the k NN ($k = 20$) algorithm to estimate the conditional entropy $H(S|R)$, where R is the quantified response, and obtain $I(S,R) = H(S) - H(S|R)$. Then, by dividing the result by experiment duration, we estimated the bitrate, see [Materials and methods](#). As in the reconstruction-based approach, we used an 8-min rolling window. As shown in [Fig 4A and 4B](#), the reconstruction-free estimates are about 2 bit/h higher than those obtained in the reconstruction-based approach, and equal (mean $\pm$ standard error of the mean):

- 8.2 ± 0.1 bit/h for the interval encoding protocol (based on four data points encircled in [Fig 4A](#)) and
- 8.5 ± 0.2 bit/h for the interval encoding protocol with a minimal gap (based on six data points encircled in [Fig 4B](#)).

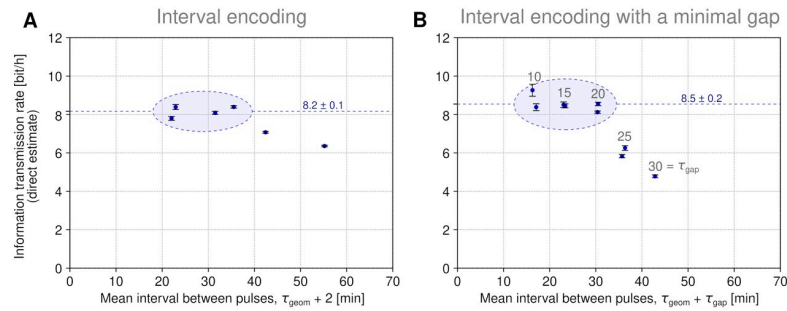


Fig 4. Channel capacity estimation—reconstruction-free approach. (A)–(B) Information transmission rate for two families of interval encoding protocols. Error bars indicate the standard deviation of the estimates obtained after 10 random resamplings of 50% (200 out of 400) cell trajectories. The maximum bitrate for a given protocol is estimated as the mean $\pm$ standard error of the mean of 4 or 6 encircled points and marked with a dashed line.

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The largest difference between the reconstruction-based and the reconstruction-free approach can be observed for data points corresponding to the shortest mean intervals (*cf.* Figs 3B and 4A). Heuristically, it can be explained as follows: In the reconstruction-based approach, the small missed pulse errors (occurring for pulses preceded by long intervals) are averaged with large missed pulse errors (occurring for pulses preceded by short intervals). Thus, for short mean intervals, the average fidelity of the pulse reconstruction is relatively low which implies high $H(S|R)$ calculated from the confusion matrix. To illustrate this with a simple example: when one sends 20 zero-one digits, and the first 10 of them are received with $p = 1$ confidence, while remaining with $p = \frac{1}{2}$ confidence, then the transmitted information is 10 bits (provided that the receiver knows which bits should be trusted). However, when the receiver does not know which bits should be trusted, the averaged confidence is $p_a = \frac{3}{4}$, which implies that the transmitted information is $20 (1 - H(S|R)) = 20 (1 + p_a \log_2(p_a) + (1 - p_a) \log_2(1 - p_a)) \approx 3.8$ bit. In the reconstruction-free approach the uncertainty about pulses is not averaged.

Discussion

Toettcher *et al.* [27] showed that the RAS–ERK module of the MAPK pathway may transmit input signals across a broad range of timescales, from 4 min to multiple hours. Here, by applying signal reconstruction-based approach we estimated the information transmission rate from FGFR to an ERK activity reporter to find that the MAPK/ERK channel capacity exceeds 6 bit/h. In a direct, reconstruction-free approach, we obtained a higher estimate of 8.5 bit/h, which shows that at the signal reconstruction step a substantial portion of information is lost. It is debatable, however, whether cells can utilize the input information without “reconstructing the signal”.

We analyzed three protocols to encode information: binary encoding, interval encoding, and interval encoding with a minimal gap, to find that interval-encoded information can be transmitted at a significantly higher bitrate than binary-encoded information. The presence of a minimal gap between pulses of length comparable to the refractory time (*i.e.*, time in which the cell may not unambiguously respond to a subsequent pulse) further increases the bitrate (although this increase is small for the reconstruction-free approach). The lower bound of the MAPK/ERK channel capacity found in this study is well above our earlier purely theoretical estimates [28], which suggested a bitrate between 1.4 and 1.9 bit/h. Those MAPK-model estimates were obtained for the binary protocol, but the main source of discrepancy is the longer

refractory time of ~50 min, arising in the computational model [29]. Interval encoding appears the most natural from the biological perspective as it assumes that pulses occur independently with some constant intensity (the mean interval is the inverse of the intensity). These random pulses can be associated with single-cell apoptosis, which recently has been found to trigger synchronous ERK activation [30] that propagates radially for about three cell “layers” [17].

Using the reconstruction-based approach we found that for the interval encoding with a minimal gap the bitrate may exceed 6 bit/h, which can be considered as a “safe” lower bound on the MAPK/ERK channel capacity. The fact that interval encoding is associated with the highest bitrate follows from the cells’ ability to respond to light pulses in a very synchronous manner. The light pulses can be detected nearly perfectly with one-minute resolution (provided that the time from the preceding pulse is longer than the refractory time), based on the ERK activity in an 8-min interval ending 5 min after the light pulse. Synchronous responses to the stimuli may enable long-distance waves of ERK activity that assist wound healing, inducing collective cell migration [18,31]. In our experiments we do not observe communication between cells, because all cells are illuminated equally and simultaneously. However, when cells are stimulated in a single spot only, for example due to an apoptotic event, one may observe propagation of an ERK activity wave outside of this spot [17,32]. This clearly indicates for cell-to-cell communication and propagation of information. One could expect that even if such wounding- or apoptosis-generated waves are initiated at small time intervals, the first “layer” of cells filters out the pulses that occur within the refractory time, and thus the farther “layers” of cells will be subjected to stimulation that resembles the interval encoding with a minimal gap.

Since we have not investigated signaling with resolution finer than 1 min we may not rule out that the MAPK/ERK channel capacity exceeds the determined lower bound of 6 bit/h. Using finer temporal discretization would increase the entropy of the input, but in the reconstruction-based approach it would also result in more classification errors, therefore both $H(S)$ and $H(S|R)$ would be increased. Even for one-minute resolution, a substantial part of information loss is due to inaccurate detections (see Fig 2D and 2F).

In our analysis we lumped together responses of different cells. This is known to reduce the measured information transfer because cell-to-cell differences in responses are treated as noise. As observed earlier by Toettcher *et al.* [27], individual cells exhibit precise and reversible Ras/ERK dose-response behavior; in our case we observe that there are faster and slower cells, and this causes that we occasionally reconstruct pulses with 1 minute delay or 1 minute in advance. By learning about each individual cell from its response to first light pulses, one could use its response to reconstruct subsequent pulses with higher accuracy; such type of analysis was performed by Selimkhanov *et al.* [7]. However, when one aims at interpreting transmitted information as the ability of cells to exhibit synchronous responses to stimuli (required for ERK activity wave propagation), cell-to-cell differences can be interpreted as an (extrinsic) noise.

Cells send, receive, and process information, however a natural question is how the information transmitted at the rate exceeding 6 bit/h can be utilized. Intuitively, high information rate is critical when a cell has to respond to constantly changing stimuli. Neurons were shown to transmit information at rates of tens of bits per second [13,14]. *E. coli* chemotactic system transfers about 0.01 bit per second, and this information rate limits its drift velocity in shallow chemical gradients [15]. Meier *et al.* [16] showed that *D. discoideum* (chemotaxis of which is known to be regulated by an atypical MAP kinase, ERKB [33,34]) may navigate in a switching chemotactic gradient, changing its direction within a minute. This suggests the information processing rate of about 1 bit per minute, or less if some cell switch-or-not decisions are incorrect.

In higher organisms, the MAPK/ERK pathway is known to coordinate diverse complex behaviors and cell fate decisions, such as cell proliferation, differentiation, migration, senescence and apoptosis [35]. The distinction between particular cell fates depends on ERK pulsatile activity integrated over timescales of multiple hours [36]. RAS-to-ERK cascade transmits signals (and information) from numerous inputs to numerous outputs regulated by ERK and its upstream components, MEK, RAF, and RAS. During collective cell migration, ERK controls myosin contractile pulses [37]. Spatially localized RAS activation is responsible for formation of actin-based cell protrusions [38], which arise and contract at the timescale of single minutes [39], directing cell motion. Since ERK works as a RAS-activated (via RAF and MEK) RAS inhibitor [40], the ERK activity profile influences cell protrusion and retraction, and thus cell motion [41]. The high information capacity of the MAPK/ERK channel demonstrated in this study enables coordination of the versatile functions associated with ERK and its upstream kinases, with cell motion in response to rapidly changing stimuli likely being the process the most information is fed into.

Materials and methods

Cell lines

The MCF-10A human mammary epithelial cells (derived from an adult female) were a gift of J.S. Brugge. The cells were modified to stably express the nuclear marker H2B-miRFP703, the ERK biosensor ERK-KTR-mRuby2 [42] and the optogenetic actuator optoFGFR [23] and used before in Refs. [17,25].

Cell culture

The modified MCF-10A cells were cultured in tissue-culture treated plastic flasks and fed with a growth medium composed of DMEM:F12 (1:1) supplemented with horse serum 5%, recombinant human EGF (20 ng/ml, Peprotech), L-glutamine, hydrocortisone (0.5 µg/ml, Sigma-Aldrich/Merck), insulin (10 µg/ml, Sigma-Aldrich/Merck), penicillin (200 U/ml) and streptomycin (200 µg/ml). The cells were routinely split upon reaching ~90% confluency. For time-lapse optogenetic experiments, cells were seeded on 24-well 1.5 glass bottom plates (Cellvis) coated with 5 µg/ml fibronectin (PanReac AppliChem) at 1×10^5 cells/well density in growth medium two days before the experiment. Four hours before the optogenetic stimulation (2.5 h before imaging), cells were washed twice with PBS and then cultured in a starvation medium composed of DMEM:F12 (1:1) supplemented with BSA (0.3% Sigma-Aldrich/Merck), L-glutamine, hydrocortisone (0.5 µg/ml, Sigma-Aldrich/Merck).

Optogenetic stimulation

OptoFGFR was stimulated with pulses of blue LED light (100 ms, 470 nm, 3 W/cm^2) applied according to specific stimulation protocols (see a further subsection on stimulation protocols). It should be noticed that for short pulses the response depends on the total pulse energy per unit surface, and that 0.3 J/cm^2 is above the saturation dose [25].

Imaging

Imaging experiments were performed on an epifluorescence Eclipse Ti inverted fluorescence microscope (Nikon) controlled by NIS-Elements (Nikon) with a Plan Apo air 20× (NA 0.8) objective. Laser-based autofocus was used throughout the experiments. Image acquisition was performed with an Andor Zyla 4.2 plus camera at the 16-bit depth every 1 min. The following excitation and emission filters were used: far red: 640 nm, ET705/72m; red: 555 nm, ET652/

60m; green: 470 nm, ET525/36m. Imaging started 1.5 h before the onset of optogenetic stimulation (to provide proper history for ERK KTR track normalization, see a further subsection on signal processing).

Stimulation protocols

In the case of binary encoding, all stimulation protocols have 19 temporally equidistant pulse “slots”. We applied the following fixed pattern of pulses: 1011010011110000101 (1 = pulse, 0 = no pulse), which is the shortest sequence containing uniquely every possible subsequence of four binary digits. For each “clock period” (i.e., time between pulse slots) this sequence (in one field of view) and its logic negation (in another field of view) were used. Since the clock periods ranged from 3 min to 30 min, overall protocol durations ranged from $19 \times 3 = 57$ to $19 \times 30 = 570$ min. The estimated values of the information transmission rate are means computed for tracks obtained from 2 fields of view and 4–5 biological replicates for each clock period, except for the clock periods of 20 min and 30 min, for which 10 fields of view from a single biological replicate were used.

In the case of interval encoding, the inter-pulse intervals were chosen in a randomized manner: the interval lengths were first selected to best reflect the underlying distribution, either geometric or geometric with a minimal gap, given the time budget of a single experiment (~26 h), and then randomly shuffled. A four hour-long resting period was added in the middle of the experiment to allow the cells to regenerate in 3 of 6 experiments with stimulation according to the protocol without a gap and 5 of 9 experiments with stimulation according to the protocol with a minimal gap; however, no significant difference was observed between results of the experiments with and without the resting period. The resting period and the initial 90-min starvation period were excluded from the analysis. The first pulse of the sequence and the first pulse after the resting period were also discarded as non-representative. Overall, in each protocol there were 24–131 analyzed pulses (during 25–31 h) depending on the assumed average interval between pulses.

Nuclei detection and cell tracking

The nuclei were detected in the channel of fluorescently tagged histone 2B (H2B)-miRFP703 using local thresholding. Outlines of overlapping nuclei were split based on geometric convexity defects when possible. Outlines of nuclei that were partially out of frame were excluded from analysis. The nuclei were tracked automatically using a greedy algorithm based on parameters such as proximity of outlines in subsequent time points, their surface area, eccentricity, orientation, total fluorescence intensity and intensity distribution. ERK KTR trajectories were obtained by calculating within each tracked nuclear outline the mean intensity in the ERK KTR channel. All image processing was performed within our custom software, SHUTTLETRACKER.

Signal processing

For each tracked nucleus, the mean ERK KTR intensity in its contour was quantified and normalized, first with the whole-image mean intensity in the ERK KTR channel (to compensate for possible global changes in fluorescence), and then with the average over the cell 120-min history (to account for variation in ERK KTR expression or its uneven visibility in individual cells). Such normalized values were subtracted from 1 and then referred to simply as ‘ERK KTR translocation’ and denoted x_t , where t indicates a time point and directly corresponds to the minute of the imaged part of the experiment. Of note, for nuclear ERK KTR signal constant in time, such normalization and linear transformation would imply $x_t = 0$.

Selection of tracks

In each field of view, we ranked tracks by their length and then by their quality, defined as low variation of the nuclear area within the track, and preselected 500 top scoring tracks. To eliminate cells that responded weakly to stimulation, for example due to low reporter expression, we further rejected 100 tracks (20%) with the lowest sum-of-squares of the discrete derivative of the ERK KTR translocation trajectory (computed after only the first step of normalization).

Input reconstruction

Binary encoding. For each experiment, 200 out of the 400 ERK KTR translocation trajectories were randomly selected as a train set, and 200 trajectories from cells stimulated with the negated sequence as the test set. For each potential light pulse time point t_0 , referred to as “slot”, we extracted slices of $\ell = 6$ subsequent time points beginning at three different shifts with respect to t_0 : 2 min before the slot (that is, from $t_0 - 2$ to $t_0 + 3$), 1 min before the slot (from $t_0 - 1$ to $t_0 + 4$), and exactly on the time point of the slot (from t_0 to $t_0 + 5$). For each slice we computed $\ell - 1$ discrete backward derivatives, $\Delta x_t = x_t - x_{t-1}$. Separately for each of the three sets of slices (corresponding to a specific shift with respect to t_0), a kNN ($k = 20$) classifier [43] with $(\ell - 1)$ -dimensional Euclidean distance was trained and evaluated so that each slice in the test set was labeled with 0/1 depending on the predicted occurrence of a light pulse in its slot. In this way, three 0/1 labels were assigned to a single slot. To obtain input signal reconstruction in each slot of x_t , an ensemble classifier combined three binary predictions through hard voting, *i.e.*, pulse is confirmed if two or three predictions are ‘yes’. In the estimation of the confusion matrix (described further), the whole procedure was repeated for 10 random resamplings of the training set and the test set. We checked that the reconstruction is not improved for higher k .

Interval encoding. The ERK KTR translocation trajectories from each experiment were split into the training set (200 randomly selected trajectories) and the test set (200 remaining trajectories). Each trajectory was represented as a set of overlapping slices of $\ell = 6$ subsequent time points. The slices constitute a perfect 6-fold coverage meaning that each (non-terminal) time point belongs to 6 (partially overlapping) slices. Each slice was labeled with the “time after pulse” (TAP) being the time from the last light pulse to the last time point of the slice (or from the previous pulse if this time is shorter than 3 min); this adjustment is important in the case of very short, 3–4 min, inter-pulse intervals). For each slice we computed $\ell - 1$ discrete backward derivatives, $\Delta x_t = x_t - x_{t-1}$. A kNN ($k = 20$) classifier [43] with $(\ell - 1)$ -dimensional Euclidean distance was trained to predict a TAP associated with each slice. Slices with TAP values of 3, 4, or 5 min were used to predict pulses through hard voting: time points indicated by at least two of three slices were considered as time points having a light pulse in the final reconstruction (see S2 Fig, panels A and B). To additionally prevent a single pulse from being predicted multiple times, if more than one prediction was indicated within any three subsequent time points, all predictions except the first one were discarded. In the estimation of the contingency table (described further), the whole procedure was repeated for 10 different random partitionings of trajectories into the test set and the train set.

Let us notice that in both the binary and the interval encoding protocols, the decision whether to classify a given time point t_0 as a point containing a light pulse was made based on 3 subsequent, partially overlapping slices of length 6, which cover 8 subsequent values of x_t (for t ranging from $t_0 - 2$ to $t_0 + 5$). Thus, only information available to a cell 5 min after the pulse was used by the trained ensemble classifier to make a prediction. In Fig 3D and 3E, the length of this window is varied to check how fast information is accumulated.

Bitrate computation

We estimated the bitrate as the amount of information $I(S, R)$ between the input signal S and the elicited response R sent within an interval corresponding to the total input duration Δt according to the formula:

$$i(S, R) = \frac{I(S, R)}{\Delta t} = \frac{H(S) - H(S|R)}{\Delta t} = \frac{H(S)}{\Delta t} - \frac{H(S|R)}{\Delta t} \quad (3)$$

where $H(S)$ is the entropy of an input signal and $H(S|R)$ is the entropy of the input conditioned on the response in the case of reconstruction-free approach or on the output-based reconstruction of the input in the case of reconstruction-based approach. As described below, the input entropy rate, $h(S) := H(S)/\Delta t$, may be determined theoretically, whereas the conditional entropy $H(S|R)$ is calculated based on ERK KTR translocation trajectories.

Input entropy rate

In the case of the binary encoding protocol, input sequences contain 0/1 digits occurring independently with identical probabilities of $\frac{1}{2}$ in temporally equidistant pulse slots. The entropy of such 19-digit input sequences is $H_{\text{binary}}(S) = 19$ bits. When digits occur in pulse slots τ_{clock} apart, then the input entropy rate per digit is

$$h_{\text{binary}}(S; \tau_{\text{clock}}) = \frac{19 \text{ bits}}{19 \tau_{\text{clock}}} = 1 \text{ bit}/\tau_{\text{clock}} \quad (4)$$

Since τ_{clock} is the clock period of the binary encoding protocol, Eq (4) expresses the input entropy rate per clock period.

In the case of the interval encoding protocols, intervals between pulses are drawn at random from the geometric distribution with the rate parameter $p = 1/\tau_{\text{geom}}$ and are optionally lengthened by adding τ_{gap} . The entropy of the geometric distribution is

$$-\frac{p \log_2 p + (1-p) \log_2 (1-p)}{p} = \tau_{\text{geom}} \log_2 \tau_{\text{geom}} - (\tau_{\text{geom}} - 1) \log_2 (\tau_{\text{geom}} - 1) \quad (5)$$

so the input entropy rate $h_{\text{interval}}(S; \tau_{\text{geom}}, \tau_{\text{gap}})$ is

$$h_{\text{interval}}(S, \tau_{\text{geom}}, \tau_{\text{gap}}) = \frac{\tau_{\text{geom}} \log_2 \tau_{\text{geom}} - (\tau_{\text{geom}} - 1) \log_2 (\tau_{\text{geom}} - 1)}{\tau_{\text{geom}} + \tau_{\text{gap}}} \quad (6)$$

where $\tau_{\text{geom}} + \tau_{\text{gap}}$ is the mean inter-pulse interval. Since in the interval encoding protocols, digits 0/1 occur at the one-minute resolution, h_{interval} computed in Eq (6) is also the entropy per digit. For the interval encoding protocol (nominally without a minimal gap), τ_{geom} was varied and a technical τ_{gap} of 2 min was introduced in experiments to avoid ambiguities in signal reconstruction. For the protocol with a minimal gap, τ_{gap} was varied and τ_{geom} was adjusted through numerical optimization to maximize $h_{\text{interval}}(S; \tau_{\text{geom}}, \tau_{\text{gap}})$.

Conditional entropy—Reconstruction-based approach

For the binary and the interval encoding protocols, we estimate from above the conditional entropy $H(S|R)$ per digit of the input given its reconstruction (throughout this section, R denotes input reconstruction). In light of Eq (3) this will provide us with a lower bound on the bitrate $i(S, R)$. Entropy of a joint distribution is lower than the sum of individual entropies

$$H(S|R) \leq \sum_d H(S_d|R) \quad (7)$$

Further, from the data processing inequality [44], for the binary encoding protocol, we have

$$H(S_d|R) \leq H(S_d|R_d) \quad (8)$$

$$H(S_d|R) \leq H(S_d|(R_{d-1}, R_d, R_{d+1})) \quad (9)$$

Taken together, from inequalities (7)–(9) we have

$$H(S|R) \leq \sum_d H(S_d|R_d) \text{ and } H(S|R) \leq \sum_d H(S_d|(R_{d-1}, R_d, R_{d+1})) \quad (10)$$

which means that to obtain upper bounds on conditional entropies for the binary encoding and the interval encoding protocols we have to calculate $H(S_d|R_d)$ and $H(S_d|(R_{d-1}, R_d, R_{d+1}))$, respectively.

Calculation of $H(S_d|R_d)$ for binary encoding. To obtain $H(S_d|R_d)$, we calculated the confusion matrix between S_d and R_d :

$$S_d = \begin{matrix} & \begin{matrix} R_d \\ 1 \quad 0 \end{matrix} \\ \begin{matrix} 1 \\ 0 \end{matrix} & \begin{matrix} TP & FN \\ FP & TN \end{matrix} \end{matrix} \quad (11)$$

where TP, FP, FN, TN are, respectively, the probabilities of true positive detections, false positive detections (“false detections”), false negative detections (“missed pulses”), and true negative detections averaged over all time points, all selected tracks, and all partitions of data into the train set and the test set. Based on this confusion matrix we calculated $H(S_d|R_d)$ according to the definition as:

$$H(S_d|R_d) = -E(\log_2 p(S_d|R_d)) = -\sum_{s,r \in \{0,1\}} p(S_d = s, R_d = r) \log_2 \frac{p(S_d = s, R_d = r)}{p(R_d = r)} \quad (12)$$

Calculation of $H(S_d | (R_{d-1}, R_d, R_{d+1}))$ for interval encoding. For this encoding, we computed the contingency table showing the relation between S_d and the reconstruction of the three subsequent digits (R_{d-1}, R_d, R_{d+1}) , which also accounts for information carried by

inaccurate detections:

$$\begin{array}{ccccc}
 & & (R_{d-1}, R_d, R_{d+1}) \\
 & & (0, 0, 0) \quad (1, 0, 0) \quad (0, 1, 0) \quad (0, 0, 1) \\
 S_d & \begin{array}{c} 1 \\ 0 \end{array} & \begin{array}{c} P_{(0,0,0);1} \\ P_{(0,0,0);0} \end{array} & \begin{array}{c} P_{(1,0,0);1} \\ P_{(1,0,0);0} \end{array} & \begin{array}{c} P_{(0,1,0);1} \\ P_{(0,1,0);0} \end{array} & \begin{array}{c} P_{(0,0,1);1} \\ P_{(0,0,1);0} \end{array}
 \end{array} \quad (13)$$

The entries of the contingency table are joint probabilities $p_{(r_-, r_0, r_+);s} = p(S_d = s, (R_{d-1}, R_d, R_{d+1}) = (r_-, r_0, r_+))$ of input signal $s \in \{0, 1\}$ and reconstructed input $(r_-, r_0, r_+) \in \{(0,0,0), (1,0,0), (0,1,0), (0,0,1)\}$. In contrast to the confusion matrix for the binary encoding, these joint probabilities have no straightforward interpretation in terms of TP, FP, FN, TN or inaccurate detection probabilities. The combinations of (R_{d-1}, R_d, R_{d+1}) containing two or three 1s do not occur due to the prior elimination of detections that are closer than 3 min apart. The conditional entropy was calculated according to the definition as:

$$H(S_d | (R_{d-1}, R_d, R_{d+1})) = - \sum_{s, r_-, r_0, r_+ \in \{0,1\}} p_{(r_-, r_0, r_+);s} \log_2 \frac{p_{(r_-, r_0, r_+);s}}{p_{(r_-, r_0, r_+)}} \quad (14)$$

To summarize, bitrate may be computed based on Eq (3) with input entropy rates given by Eqs (4) and (6) and conditional entropies given by Eqs (12) and (14) in the case of the binary encoding protocols and in the case of interval encoding protocols, respectively.

Reconstruction-based approach—Sources of information loss

To determine the sources of the information loss (Fig 2B, 2D and 2F), we sequentially corrected all types of errors in the reconstruction: false detections (false positives), missed pulses (false negatives), and for the interval encoding protocols also inaccurate (deferred or advanced) detections. After each correction step, we recomputed the confusion matrix/contingency table and attributed the decrease of conditional entropy and the resulting increases of bitrate to the particular type of error. Since the obtained results depend on a particular order of correction steps, to compare individual contributions from the three corresponding types of reconstruction errors, we calculated each of these contributions by averaging over all possible orders of the correction steps (2 permutations for the binary encoding protocol; 6 permutations for the interval encoding protocols; see S2 Fig, panels C–E). Of note, each sequence of correction steps restores exactly the input signal and the estimated bitrate losses associated with considered error types sum up to the total bitrate loss. Since the procedure is symmetric with respect to each type of error, these contributions may be compared.

Conditional entropy—Reconstruction-free approach

To estimate the information transmission rate directly, *i.e.*, without the reconstruction step (Fig 4), we split the trajectories into slices of length $\ell = 8$ as described above and labeled them with 0/1 depending on whether an input pulse occurred at the time point 5 min before the last time point in the slice. The slice length $\ell = 8$ was chosen to comply with the reconstruction-based approach, where we used three consecutive slices of length 6 that jointly span over an interval of 8 time points. Seven ($= \ell - 1$) finite differences were computed for each slice, r , embedding all slices in the 7-dimensional Euclidian space. For each r , $k = 20$ nearest neighbors were found (including the slice itself) and the numbers n_0 and n_1 of neighbors marked with 0 and 1, respectively, were counted ($n_0 + n_1 = k$). For each slice, the conditional entropy was

estimated according to the formula:

$$H(S|r) = -\left(\frac{n_0}{k} \log_2 \frac{n_0}{k} + \frac{n_1}{k} \log_2 \frac{n_1}{k}\right) + \text{bias correction} \tag{15}$$

where (Miller–Madow) bias correction is $1/(2k \ln 2)$ if both n_0 and n_1 are non-zero, and 0 otherwise [45,46]. The overall conditional entropy $H(S|R)$ was estimated by averaging $H(S|r)$ over all slices r . The procedure was performed on 200 trajectories randomly chosen out of 400 cell trajectories in each experiment, and repeated 10 times for different choices to evaluate the robustness of the estimate.

Supporting information

S1 Fig. Experimental and data analysis workflow (reconstruction-based approach). (TIF)

S2 Fig. Interval encoding: Input sequence reconstruction algorithm and determination of the sources of information loss (reconstruction-based approach). (A) Tracks from the training set are segmented into overlapping slices of 6 consecutive time points such that each time point belongs to 6 slices. Each slice is labeled with the time after pulse (TAP), measured with respect to the last time point in the slice. The k NN classifier is trained to predict the TAP based on 5 discrete differences between 6 consecutive time points of a slice. (B) For each slice, the TAP predicted by the classifier indicates a time point at which the stimulation pulse could occur. Votes for particular time points from different slices are counted. Only votes from slices predicted with TAP = 3,4,5 min are taken into account, the remaining are ignored as unreliable. Time points that received at least two out of the three possible votes are considered as time points with pulse in the final reconstruction, R . (C) Detection and labeling of errors in the reconstruction R . Inaccurate detections (one minute before or after the pulse) are not decomposed into missed pulses and false detections but assigned to their specific error types. Since the patterns ‘11’ and ‘101’ are guaranteed never to occur in the input sequence S , the error classification is unambiguous. (D) An example 3-step sequence of error corrections for the interval encoding protocols. After the third step, the fully corrected reconstruction R''' is identical to the input sequence S . The difference between bitrate before and after each correction step is attributed to the particular information loss source. (E) As the difference in bitrate depends on the sequence of corrections, the contributions of the three types of errors are averaged over all permutations of correction steps. (TIF)

S3 Fig. Cell-to-cell bitrate variability and exclusion of non-responding cells (reconstruction-based approach). (A) Bitrate estimate as a function of the fraction of cells excluded in the preselection step. The (lower bound for) bitrate is computed as in Fig 3. Error bars denote standard error of the mean based on the 2–4 experiments with highest bitrate. In the preselection step we aim at excluding cells that do not respond to stimulation, possibly due to low expression of optoFGFR or ERK KTR. The rejection criterion is formulated in a way *a priori* independent of the accuracy of pulse detection, see Methods for details. Throughout the paper, the fraction of rejected cells is set to 20% (highlighted in gray), because above this value the bitrate estimates in the interval encoding protocols (with and without minimal gap) reach a plateau. (B, C, D) Histograms of the information transmission rates in single cells for (B) binary encoding, (C) interval encoding, and (D) interval encoding with a minimal gap. Estimates for cells rejected in the preselection step (20% of all cells) are marked in orange. Negative

bitrate estimates can occur due to the rough approximation based on inequalities in Eq (7) in the main text.

(TIF)

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Supporting Information

The MAPK/ERK channel capacity exceeds 6 bit/hour

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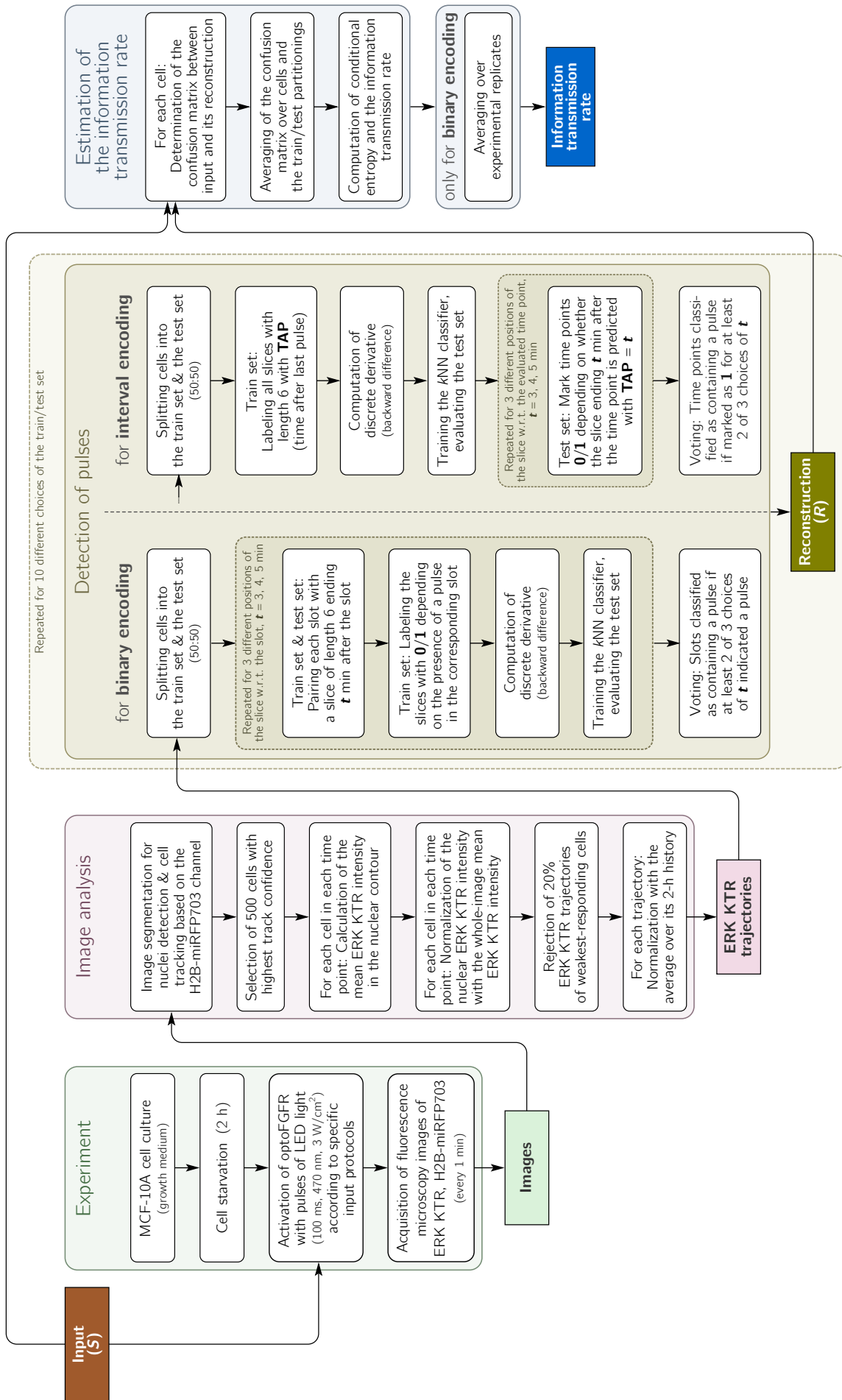
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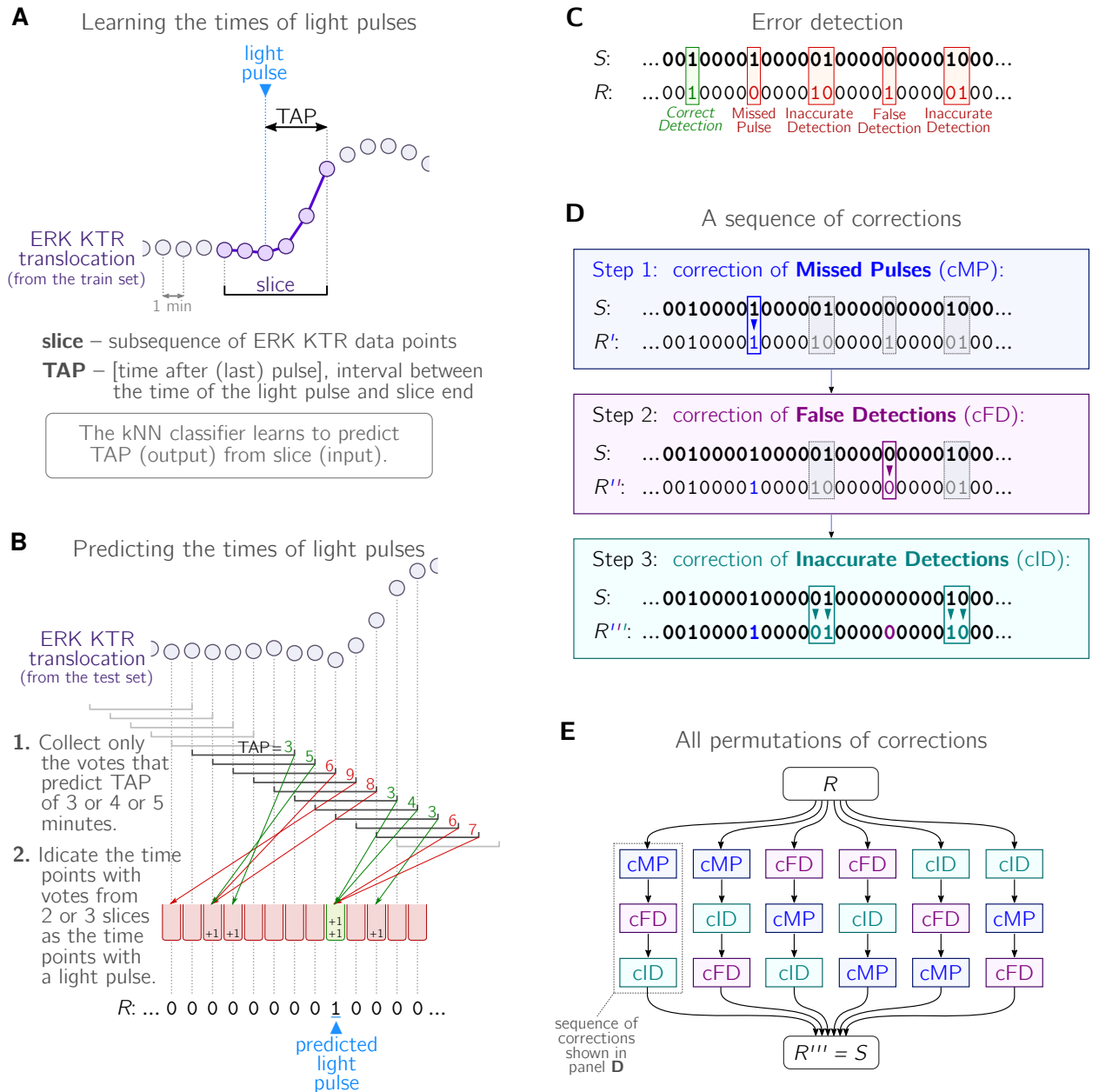
Other supporting materials for this article include:

Source data at DOI: [10.5281/zenodo.7808385](https://doi.org/10.5281/zenodo.7808385)

Source code at URL: <https://github.com/pawelnaiecz/pulsatile-information>



S1 Fig. Experimental and data analysis workflow (reconstruction-based approach).



S2 Fig. Interval encoding: input sequence reconstruction algorithm and determination of the sources of information loss (reconstruction-based approach).

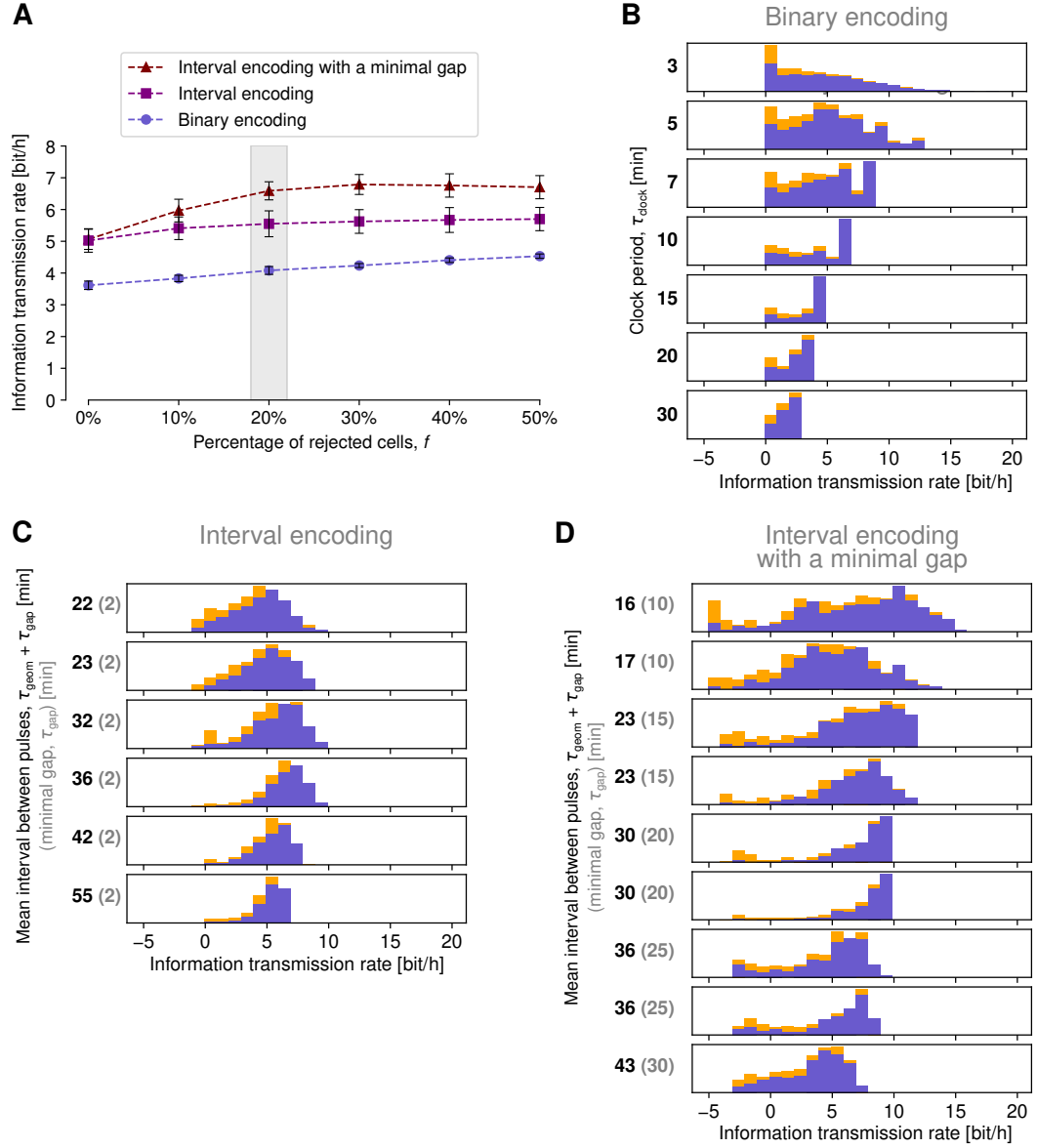
(A) Tracks from the training set are segmented into overlapping slices of 6 consecutive time points such that each time point belongs to 6 slices. Each slice is labeled with the time after pulse (TAP), measured with respect to the last time point in the slice. The kNN classifier is trained to predict the TAP based on 5 discrete differences between 6 consecutive time points of a slice.

(B) For each slice, the TAP predicted by the classifier indicates a time point at which the stimulation pulse could occur. Votes for particular time points from different slices are counted. Only votes from slices predicted with TAP = 3, 4, 5 min are taken into account, the remaining are ignored as unreliable. Time points that received at least two out of the three possible votes are considered as time points with pulse in the final reconstruction, R .

(C) Detection and labeling of errors in the reconstruction R . Inaccurate detections (one minute before or after the pulse) are not decomposed into missed pulses and false detections but assigned to their specific error types. Since patterns of '11' and '101' are guaranteed never to occur in the input sequence S , the error classification is unambiguous.

(D) An example 3-step sequence of error corrections for the interval encoding protocols. After the third step, the fully corrected reconstruction R''' is identical to the input sequence S . The difference between bitrate before and after each correction step is attributed to the particular information loss source.

(E) As the difference in bitrate depends on the sequence of corrections, the contributions of the three types of errors are averaged over all permutations of correction steps.



S3 Fig. Cell-to-cell bitrate variability and exclusion of non-responding cells (reconstruction-based approach).

(A) Bitrate estimate as a function of the fraction of cells excluded in the preselection step. The (lower bound for) bitrate is computed as in Figure 3. Error bars denote standard error of the mean based on 2–4 experiments with the highest bitrate. In the preselection step we aim at excluding cells that do not respond to stimulation, possibly due to low expression of optoFGFR or ERK KTR. The rejection criterion is formulated in a way *a priori* independent of the accuracy of pulse detection, see Methods for details. Throughout the paper, the fraction of rejected cells is set to 20% (highlighted in gray), because above this value the bitrate estimates in the interval encoding protocols (with and without minimal gap) reach a plateau.

(B, C, D) Histograms of the information transmission rates in single cells for (B) binary encoding, (C) interval encoding, and (D) interval encoding with a minimal gap. Estimates for cells rejected in the preselection step (20% of all cells) are marked in orange. Negative bitrate estimates can occur due to the rough approximation based on inequalities in Eq. (8) in the main text.



OPEN Quantifying cancer- and drug-induced changes in Shannon information capacity of RTK signaling

Paweł Nałęcz-Jawecki^{1,4}, Lee Roth^{2,4}, Frederic Grabowski¹, Sunnie Li², Marek Kochańczyk¹, Lukasz J. Bugaj²✉ & Tomasz Lipniacki^{1,3}✉

Cancer can result from abnormal regulation of cells by their environment, potentially because cancer cells may misperceive environmental cues. However, the magnitude to which the oncogenic state alters cellular information processing has not been quantified. Here, we apply pseudorandom pulsatile optogenetic stimulation, live-cell imaging, and information theory to compare the information capacity of receptor tyrosine kinase (RTK) signaling pathways in EML4-ALK-driven lung cancer (STE-1) and in non-transformed (BEAS-2B) cells. The average information rate through RTK/ERK signaling in STE-1 cells was less than 0.5 bit/hour, compared to 7 bit/hour in BEAS-2B cells, but increased to 3 bit/hour after oncogene inhibition. Information was transmitted by 50–70% of cells, whose channel capacity (maximum information rate) was estimated through in silico protocol optimization. In BEAS-2B cells, channel capacity of the parallel RTK/calciueurin pathway surpassed that of the RTK/ERK pathway. This study highlights information capacity as a sensitive metric for identifying disease-associated dysfunction and evaluating the effects of targeted interventions.

Keywords Channel capacity, MAPK pathway, Cancer, EML4-ALK oncogene, Optogenetics

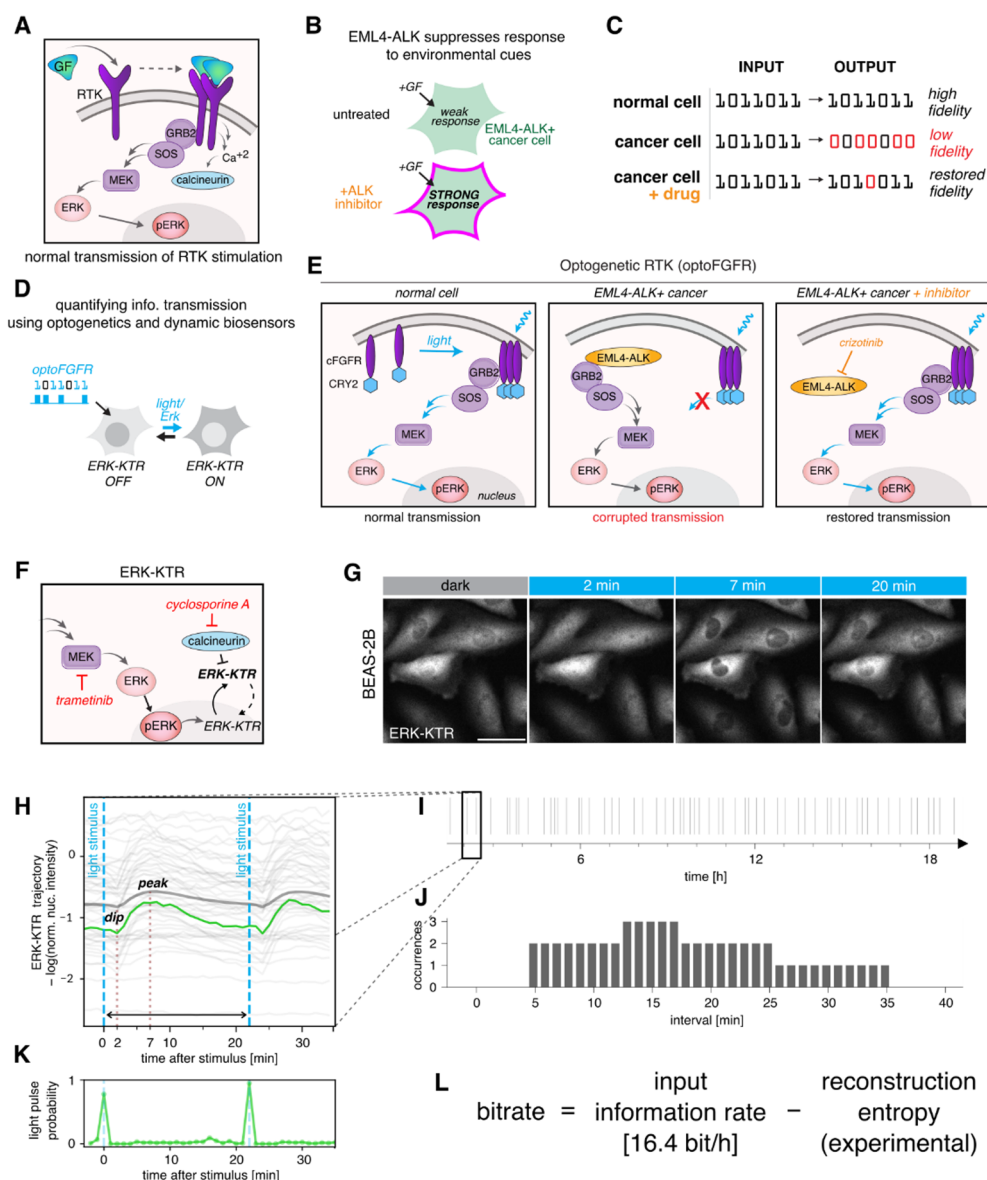
The existence of oncogenes throughout healthy human tissues underscores decades of studies showing that cancer arises not only from the presence of an oncogene but also from improper regulation of precancerous cells by their environment^{1,2}. Such misregulation may result not only from changes in the cell's environment but also from changes in how a cell responds to cues from that environment. However, how an oncogenic state alters a cell's perception of its environment remains largely unexplored.

Biochemical signaling pathways regulate a cell's response to its environment. For example, ligand stimulation of receptor tyrosine kinases (RTKs) triggers the RAS/RAF/MEK/ERK pathway (Fig. 1A), referred to here as the RTK/ERK pathway, which is responsible for cell proliferation, survival, and differentiation³. The RTK/ERK pathway is often stimulated in transient pulses and regulates processes that require high spatiotemporal resolution, such as collective cell migration and embryogenesis^{4–6}. RTK signaling also activates additional pathways, including the PI3K/AKT/mTOR and calcium signaling pathways, both of which are also dynamic and important for cell growth and division^{7–10}. In line with the central role of RTKs in cell proliferation, mutations in RTKs and downstream pathways are frequently associated with cancer.

Although typically studied for their effects on the amplitude of tonic signaling, cancer mutations can disrupt additional features of signal transmission, including its kinetics or sensitivity, which may alter the cells' ability to respond to extracellular signals appropriately^{11–13}. One example is the EML4-ALK fusion oncoprotein^{14,15}, which drives constitutive activation of ERK signaling but simultaneously suppresses signaling through RTKs, reducing the cell's response to external growth factors. These disruptions can be reversed by targeted drugs, which restore responsiveness to growth factors and permit pulsatile ERK activity (Fig. 1B)¹². Although examples of oncogene-induced changes in signal transmission continue to emerge, it is not clear to what extent cancer alterations corrupt a cell's capacity to perceive its environment because changes in information transmission through dynamic signaling networks have not been quantified.

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Shannon information theory¹⁶ provides a framework for the quantitative assessment of the rate at which information can be transferred through a communication channel, such as a signaling pathway. While *mutual information* measures the information about the input signal that can be inferred from the observed response, the *information transmission rate* (or *bitrate*) reflects the amount of information transferred through the channel per unit time. The maximum rate at which information can be transmitted through a channel using the best encoding is called the *channel capacity*. Early studies used mutual information in population-level measurements to find that the response amplitude in several important signaling pathways, including the RTK/ERK pathway, transmits only approximately 1 bit of information about the strength of a stimulus, indicating an all-or-nothing response^{17,18}. Subsequent works showed that slightly more information can be transmitted when accounting for the temporal pattern of the response¹⁹. A recent study examined repetitiveness of ERK activation in single cells and found that cells can transmit more than 2 bits of information²⁰, which could not be detected at the population level due to phenotypic diversity between individual cells^{21–25}. The information transmission rate is an appropriate measure in systems where repeated use of a channel rather than a one-time decision is important because it accounts for both the fidelity of signal transmission and its temporal resolution. In such systems, information may be encoded in the intervals between signaling pulses. Recently, we showed that such encoding allows transmission of at least 6–8 bit/h through the RTK/ERK pathway²⁵, exceeding earlier theoretical estimates²⁶.

In this study, we apply Shannon information theory together with optogenetics and live-cell imaging to quantify changes in signal transmission resulting from the expression of the EML4-ALK fusion oncoprotein in the patient-derived STE-1 cancer cell line. We show that in this cell line, information transmission through the RTK/ERK pathway is almost entirely blocked. Treatment with the ALK inhibitor (ALKi) crizotinib restored information transmission, although to a rate still twice as low as that observed in noncancerous BEAS-2B cells (Fig. 1C). In these cells, RTK-triggered calcium/calcineurin signaling transmitted even more information than the RTK/ERK pathway, although activation of the calcineurin pathway was not observed in STE-1 cells. Our

◀Fig. 1. Experimental setup. (A) After binding to a growth factor (GF), receptor tyrosine kinases (RTKs) multimerize and signal to ERK via RAS, RAF, and MEK (the RTK/ERK pathway). Simultaneously, RTKs trigger the calcium-calmodulin pathway (RTK/calmodulin pathway). (B) The EML4-ALK oncogene suppresses a cell's ability to respond to environmental cues such as growth factors. The ALK inhibitor (ALKi) restores responsiveness. (C) In normal cells, activity pulses are accurately transmitted by the RTK/ERK pathway from receptors to the nucleus. EML4-ALK-positive cancer cells transmit environmental information with low fidelity, which can (to some extent) be restored by ALKi. (D) Pulsatile optogenetic FGFR (optoFGFR) stimulation and fluorescent biosensors of signaling allow the quantification of information transmission through the RTK/ERK signaling axis. The ERK-KTR biosensor translocates between the nucleus and the cytoplasm upon ERK activation/inactivation. (E) OptoFGFR, in response to blue light, recapitulates endogenous RTK activation. Cell responsiveness to RTK signals may be suppressed by EML4-ALK, which associates with and sequesters GRB2-SOS. Signal transmission can be restored by ALKi, which liberates GRB2-SOS from EML4-ALK. (F) ERK-KTR migrates to the cytoplasm upon phosphorylation by ERK, and in the reverse direction after dephosphorylation by calcineurin. Inhibitors of calcineurin (cyclosporine A) or MEK (trametinib) were used to study the two pathways in isolation. (G) ERK-KTR cytoplasmic–nuclear shuttling after stimulation with a light pulse in BEAS-2B cells. Fluorescence was recorded with a one-minute resolution. Scale bar = 20 μ m. (H) Light stimulus leads to ERK-KTR phosphorylation, resulting in cytoplasmic translocation (a peak is observed approximately 7 min after the light pulse), preceded by a calcineurin-mediated 'dip' observed 2 min after the light pulse in BEAS-2B cells. The thin gray lines represent single-cell preprocessed ERK-KTR trajectories, and the thick gray line represents their average. The green line shows a selected single-cell trajectory for which the (posterior) probability of a light pulse is given in panel K. See Methods for details of ERK-KTR trajectory preprocessing. (I) The cells were stimulated with a pseudorandom series of light pulses. Information is encoded in intervals between subsequent pulses. (J) The distribution of intervals between stimulation pulses was chosen on the basis of preliminary experiments to maximize the bitrate. (K) Single-cell ERK-KTR trajectories were used for probabilistic reconstruction of the input signal, i.e., to predict the probability of a light pulse occurrence at each time point throughout the experiment at a one-minute temporal resolution. (L) The numerically estimated entropy of the probabilistic reconstruction was used to compute the rate of information transmission through the pathway.

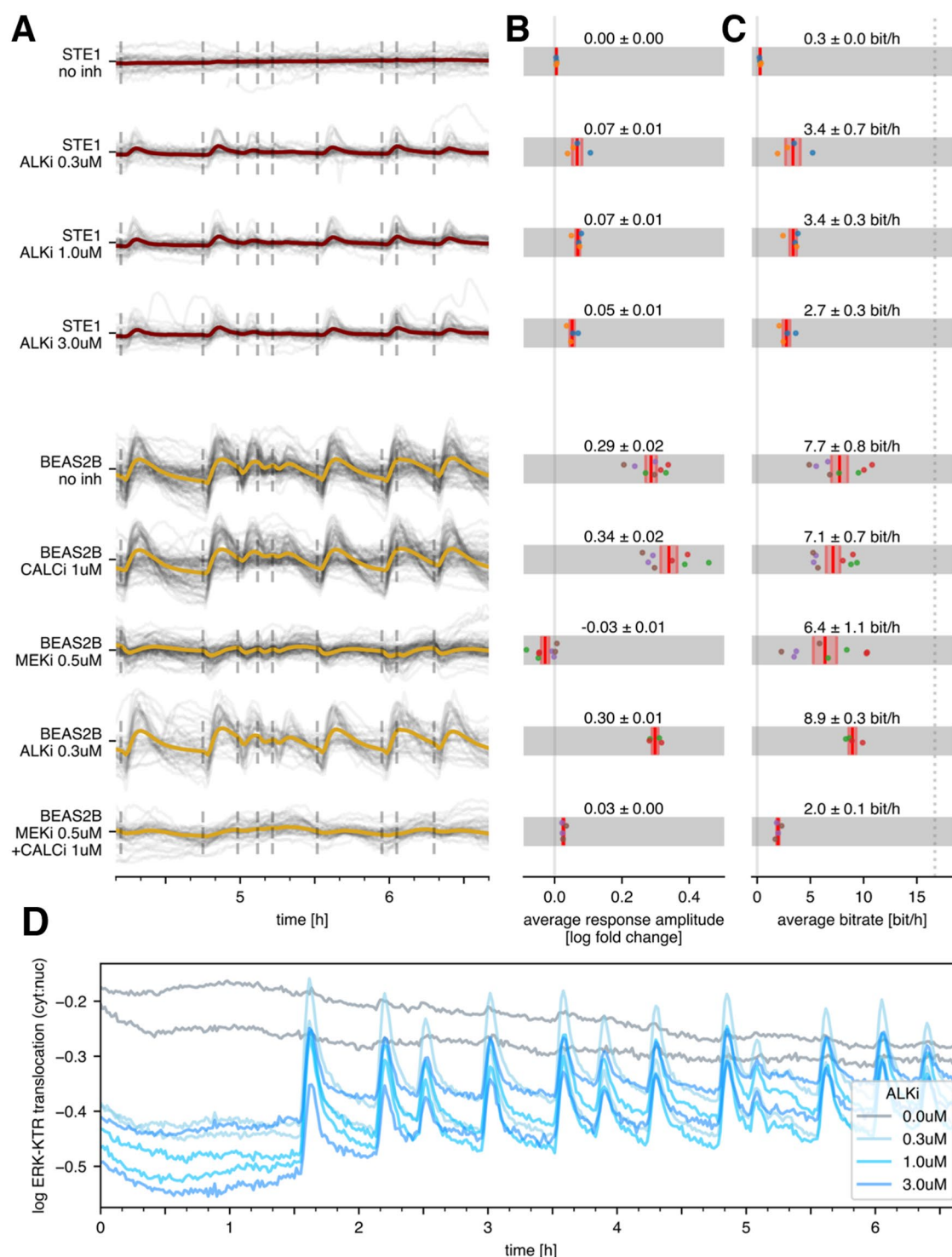
work demonstrates how information theory can provide a quantitative, functional metric to understand the oncogenic state and assess potential therapies.

Results

Pulsatile OptoFGFR stimulation enables estimation of information flow in the RTK/ERK pathway

To monitor and induce the activity of the RTK/ERK pathway, we engineered cells to express a fluorescent reporter of ERK activity (ERK-KTR) and an optogenetic FGF receptor (optoFGFR)²⁷, which allowed us to stimulate the pathway with light at precisely selected time points (Fig. 1D). OptoFGFR is a fusion of the intracellular fragment of FGFR1 fused to the light-induced clustering module Cry2²⁸. Cry2 clustering triggers activity of FGFR1 and evokes ERK pulses similar in shape and duration to those obtained by transient FGF stimulation in a microfluidic system²⁹. We investigated an EML4-ALK-expressing human non-small-cell lung carcinoma cell line, STE-1, and a noncancerous cell line, BEAS-2B, as a reference. In normal cells, such as BEAS-2B cells, optoFGFR activation triggers RTK/ERK pathway signaling, culminating in ERK phosphorylation/activation and subsequent phosphorylation of its downstream targets. In STE-1 cells, EML4-ALK hijacks GRB2-SOS, leading to constitutive ERK activation and also to active suppression of transmembrane RTKs, including optoFGFR¹². ALK inhibition relieves this suppression and restores the cells' ability to transmit RTK signals (Fig. 1E). We monitored ERK activity using ERK-KTR, which, when phosphorylated by active ERK, translocates to the cytoplasm (Fig. 1F,G). In some cell types, including BEAS-2B cells, FGFR (and optoFGFR) also triggers calcium signaling²⁷, which leads to the activation of the phosphatase calcineurin (Fig. 1A). Because ERK-KTR is engineered from a natural substrate for calcineurin (ELK-1), calcineurin can dephosphorylate and inactivate ERK-KTR, driving its nuclear localization³⁰. To disambiguate signaling via the RTK/ERK and RTK/calmodulin pathways, we performed experiments in the presence or absence of inhibitors of MEK (trametinib, MEKi) and/or calcineurin (cyclosporine A, CALCi; Fig. 1F). In a typical experiment, 100 ms light pulses were administered at prespecified intervals (see below), and ERK-KTR fluorescence (Fig. 1G) was recorded every minute, resulting in single-cell trajectories of ERK-KTR nuclear intensity. In BEAS-2B cells, the preprocessed ERK-KTR trajectories (see Methods for preprocessing details) typically feature a small 'dip' 2 min after stimulation, resulting from calcineurin-mediated ERK-KTR dephosphorylation. This was followed by a cytoplasmic ERK-KTR translocation peak 7 min after the light pulse, which was mediated by the RTK/ERK pathway (Fig. 1H).

To measure the rate of information transmission, we stimulated STE-1 and BEAS-2B cells with a pseudorandom series of light pulses (Fig. 1I). The intervals between subsequent light pulses followed a fixed distribution (Fig. 1J), carefully chosen on the basis of preliminary experiments to ensure both a near-optimal bitrate and sampling of a broad range of intervals. Single-cell ERK-KTR trajectories enabled a probabilistic, machine learning-based reconstruction of the input signal (Fig. 1K). We used this reconstruction to compute the entropy $H(X|Y)$ of the input conditioned on the observed response (i.e., information lost owing to the uncertainty of signal reconstruction). The amount of transmitted information is then given by $I(X; Y) = H(X) - H(X|Y)$, where $H(X)$ is the input entropy (sent information). Therefore, we computed the transmitted information rate as the input information rate minus the reconstruction entropy rate (Fig. 1L). Preliminary experiments on BEAS-2B and



STE-1 cells, along with an earlier study on MCF-10A cells²⁵, indicated that the effective refractory time of the RTK/ERK pathway, i.e., the interval at which the second pulse is detected with 50% probability, is approximately 8–9 min. The chosen interval distribution for our stimulus pulse trains included intervals from 5 to 35 min and had an input entropy rate of 16.4 bit/h. In principle, the input information rate could be higher for more frequent pulses, reaching 60 bit/h for pulses occurring every minute with a probability of 1/2. However, if light pulses were sent at intervals shorter than the refractory time, the cell responsiveness would deteriorate, increasing the reconstruction entropy.

EML4-ALK blocks information transmission, which can be restored by ALK Inhibition

To estimate the reconstruction entropy (and thus the amount of information transmitted by the sequence of light pulses), we trained a multilayer perceptron (MLP with 3 layers) using all single-cell tracks longer than

Fig. 2. Response amplitude and bitrate in the STE-1 (cancerous) vs. BEAS-2B (noncancerous) cell lines, with or without inhibitors. **(A)** 2.5-hour fragments of ERK-KTR trajectories obtained in experiments with STE-1 and BEAS-2B cells with or without inhibitors, as indicated. The gray lines represent single-cell trajectories, whereas the colored lines represent the population average. The dashed vertical lines indicate light pulses. **(B)** Population-averaged response amplitude measured 7 min after the light pulse (the average was taken over all the cells and light pulses; see Supplementary Fig. S2 for the response amplitude definition). **(C)** Population-averaged bitrate. The dotted line shows the input information rate of the input pulse sequence. **(D)** ERK-KTR trajectory in STE-1 cells treated with various ALKi concentrations. Each line represents an average of all cells in a single technical replicate. Note that in this panel (and only here), the ERK-KTR trajectory was computed as the log-ratio of cytoplasmic and nuclear fluorescence, which allows for visualization of higher basal ERK-KTR activity in the absence of ALKi. In panels B and C, dots correspond to experimental/technical replicates; dots of the same color denote results from the same experimental replicate; bars correspond to the mean and standard error of the mean. The cell lines and stimulation protocols correspond to those in panel A.

3 h from both cell lines, all experimental conditions and all experimental replicates (see Methods for details). To predict the probability of a light pulse at a given time (Fig. 1K), the MLP used the following features: a 7-min fragment of the ERK-KTR trajectory, the time elapsed since the previous light pulse, a measure of track variability, and information about the cell line and an optional inhibitor. The last two inputs allowed a single MLP to generalize across experiments in which cells follow qualitatively different trajectories. During training, we directly minimized the cross-entropy, which constitutes an upper bound of the conditional entropy $H(X|Y)$. By subtracting the result from the input entropy $H(X)$, we obtained a lower bound on the transmitted information $H(X; Y)$. We noticed that cells with low (or very high) expression of optoFGFR had markedly lower bitrate, and therefore we excluded them from further analysis (see Methods and Supplementary Fig. S1). We used the remaining cells (without retraining the MLP) to estimate the population-averaged bitrate at which the STE-1 and BEAS-2B cells transmit information when stimulated according to the selected protocol (Fig. 1J).

STE-1 cancer cells were minimally responsive to light stimulation (Fig. 2A, B row 1) and thus transmitted nearly no information from the activated receptor to ERK-KTR (Fig. 2C row 1), consistent with our earlier results¹². In contrast, when treated with ALKi, a significant fraction of cells evoked an ERK-KTR trajectory peak (Fig. 2A, B rows 2–4), which allowed for an average information transmission rate of ~3 bit/h (Fig. 2C, rows 2–4). The effect did not depend on the ALKi concentration in the tested range (0.3–3 μ M), which indicates that a concentration of 0.3 μ M is sufficient to maximally restore information transmission. The increase in response magnitude was due in part to a drug-induced decrease in basal signaling, which increased the dynamic range of stimulation (Fig. 2D).

We performed the same analysis for BEAS-2B cells, obtaining a 4–6 fold stronger average response and a more than twofold higher bitrate for the RTK/ERK pathway (7.2 bit/h; Fig. 2B, C, row 6), matching results obtained for MCF-10 A cells²⁵. As expected, ALKi minimally affected the bitrate of BEAS-2B cells, which is consistent with their lack of ALK kinase expression (Fig. 2C, row 8 vs. row 5).

In BEAS-2B cells, the RTK/ERK and RTK/calceineurin pathways transmit information independently

As mentioned previously, optoFGFR signals to ERK-KTR not only through ERK but also through calcineurin. The latter pathway is faster and evokes a dip (nuclear ERK-KTR translocation) in the ERK-KTR trajectory (Fig. 1H), preceding the peak (cytoplasmic ERK-KTR translocation) induced by ERK. To verify whether the high bitrate observed in BEAS-2B cells results from the MLP classifier using this dip to detect light pulses, we inhibited the RTK/calceineurin pathway with a CALCi. While CALCi eliminated the dip and slightly increased the response amplitude (Fig. 2B, row 6), it only marginally reduced the bitrate, indicating that the RTK/ERK pathway alone can transmit approximately 7.2 bit/h (Fig. 2C row 6). Similarly, after the RTK/ERK (RTK-RAS-RAF-MEK-ERK) pathway was blocked with MEKi, the RTK/calceineurin pathway was capable of transmitting approximately 6.4 bit/h. When operating simultaneously (without any inhibitors), the two pathways transmit 7.8 bit/h. As a control, we confirmed that the joint application of CALCi and MEKi prevents effective information transmission, reducing the average response amplitude tenfold and the bitrate to 2.1 bit/h.

Overall, these results show that in the noncancerous cell line, both the RTK/ERK and RTK/calceineurin pathways, separately or jointly, transmit approximately 7 bit/h when stimulated according to the chosen encoding protocol. Information transmission is fully suppressed in STE-1 cells expressing EML4-ALK but can be restored to 3 bit/h by ALKi treatment.

In information-transmitting cells, the bitrate of ALKi-treated STE-1 cells remains half that observed in BEAS-2B cells

To explore the differences between STE-1 and BEAS-2B cells in more detail, we assessed the heterogeneity of single-cell bitrate estimates within each cell line. The distributions (Fig. 3A) covered a wide range, from approximately –2.5 bit/h to 16.4 bit/h, the latter corresponding to the input protocol entropy rate.

Although a single cell cannot have a truly negative bitrate, our estimator can yield negative values because it is based on the cross-entropy between the input sequence and a neural network's predictions (see Methods). Negative estimates arise when a cell responds atypically, so that the neural network (trained on all cells) systematically misinterprets its responses. In effect, the network's predictions for such cells are worse than the prior, resulting in a net negative contribution to the overall bitrate estimate, or, equivalently, a negative single-cell bitrate estimate. For simplicity, we refer to these estimates simply as (single-cell) bitrates.

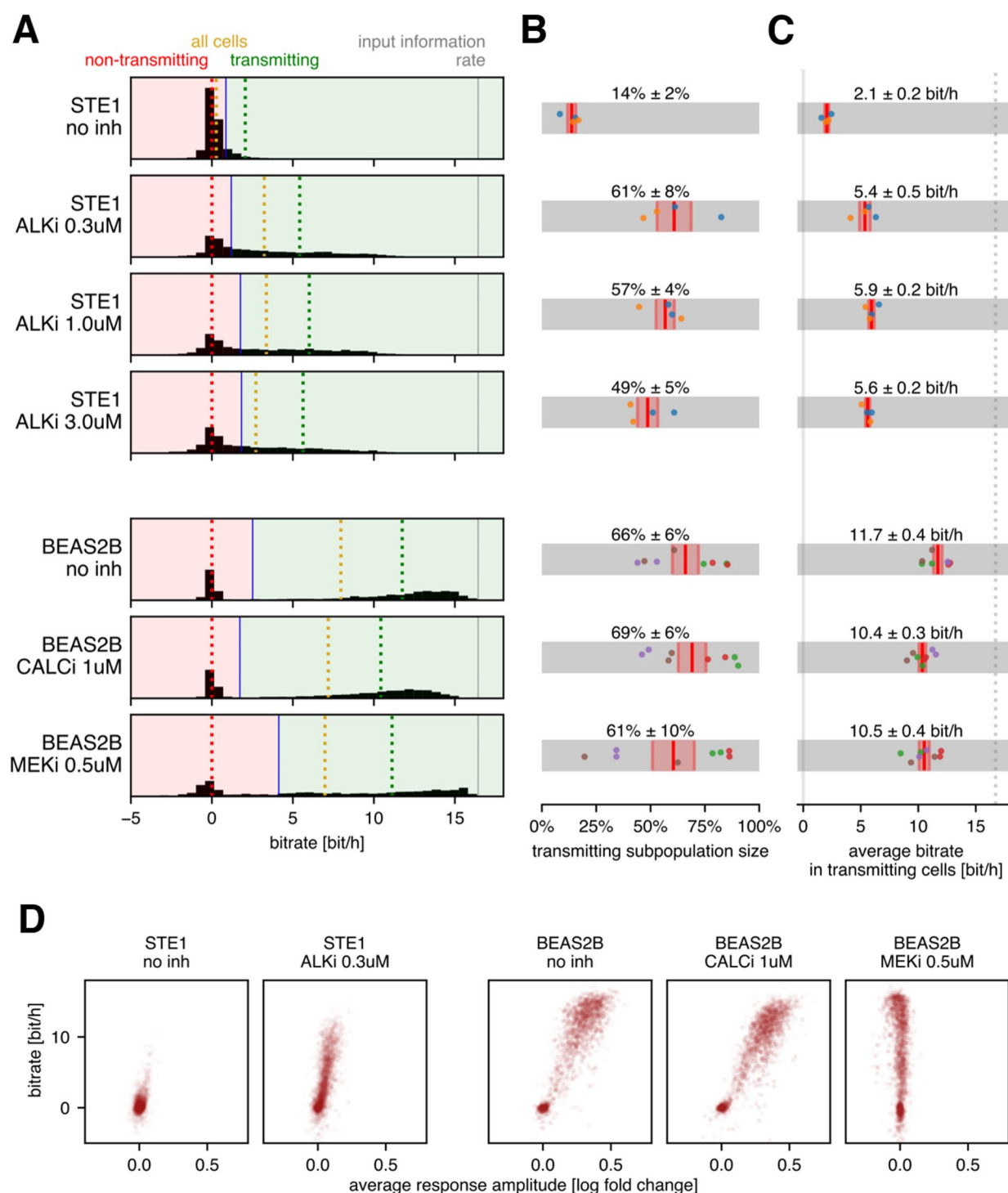


Fig. 3. Bitrate of individual cells. **(A)** Histograms of the bitrates of individual cells; for each condition, all the biological and technical replicates are pooled. The cells are divided into transmitting and non-transmitting subpopulations so that the average bitrate in the non-transmitting subpopulation is zero. The dotted lines show the bitrate averaged over non-transmitting (red), transmitting (green), and all cells (yellow). **(B)** Fractions of transmitting cells computed for each replicate separately; average and standard error of the mean are shown. **(C)** Average bitrate in the transmitting cell subpopulation computed for each replicate separately; average and standard error of the mean are shown; the dotted line indicates the input information rate. **(D)** Bitrate vs. average response amplitude in individual cells; the dot intensity is proportional to the time for which each cell was tracked. In panels B and C, dots correspond to experimental replicates; dots of the same color denote results from the same experiment. The threshold shown in panel A (blue line) was computed for all replicates pooled, whereas in panels B and C, the threshold was chosen for each replicate separately.

In BEAS-2B cells, the histograms of single-cell bitrate exhibit a distinct bimodal pattern: one group of cells clusters near zero, whereas the other centers at approximately 12–13 bit/h. In STE-1 cells with ALKi, the two modes overlap, with only a tiny fraction of cells transmitting more than 10 bit/h. This finding shows that even with ALKi treatment, STE-1 cells rarely transmit information at rates characteristic of transmitting BEAS-2B cells.

Formally, we split the cells into ‘non-transmitting’ and ‘transmitting’ subpopulations by setting a threshold on the histogram such that cells to the left of this threshold (‘non-transmitting’) transmit on average 0 bit/h. The average fraction of transmitting cells ranged between 60 and 70% for the BEAS-2B cells, regardless of the applied inhibitor, and between 50 and 60% for the ALKi-treated STE-1 cells (Fig. 3B). Untreated STE-1 cells consistently contained approximately 15% transmitting cells.

We noticed that both the average bitrate for the entire cell population (Fig. 2B) and the fraction of transmitting cells (Fig. 3B) were sensitive to experimental replicate-dependent factors. However, their ratio—the average bitrate within the transmitting subpopulation (Fig. 3C)—was much more stable. The transmitting subpopulation of STE-1 cells subjected to ALKi treatment consistently achieved a bitrate of approximately 5.5 bit/h, while the transmitting subpopulation of BEAS-2B cells reached 10–11 bit/h via either the RTK/ERK or RTK/calcineurin channel, and reached nearly 12 bit/h when neither channel was blocked by inhibitor.

We observed a clear correlation between the response amplitude and the bitrate in individual cells (Fig. 3D), indicating that the ‘non-transmitting’ cells are primarily non-responding. While mathematically bitrate does not depend on the response amplitude, it depends on pulse detectability, and thus the signal-to-noise ratio. Figure 3D suggests that the higher bitrate observed in BEAS-2B cells than in ALKi-treated STE-1 cells is due mainly to the higher average response amplitude, which allows for better separation of the ERK-KTR peak from background noise.

Importantly, while the difference in response amplitude between BEAS-2B cells and treated STE-1 cells is evident, its origin is not obvious. It may be associated with cancer- or drug-induced changes in RTK/ERK dynamics, but also with the cell type or reporter. It may as well result from richer (2% FBS) serum used for STE-1 to prevent cell death over the 17-hour experimental period.

Information-transmitting cells are spatially correlated

We observe that cells distinguished as information-transmitting tend to cluster. As shown in Fig. 4, the probability that the k th nearest neighbor of a transmitting cell transmits information is elevated with respect to neighbors of non-transmitting cells. The effect is the most visible for $k \lesssim 6$. This may indicate that sister cells have a tendency to be either both transmitting or non-transmitting, although a dedicated experiment would be necessary to confirm the statement.

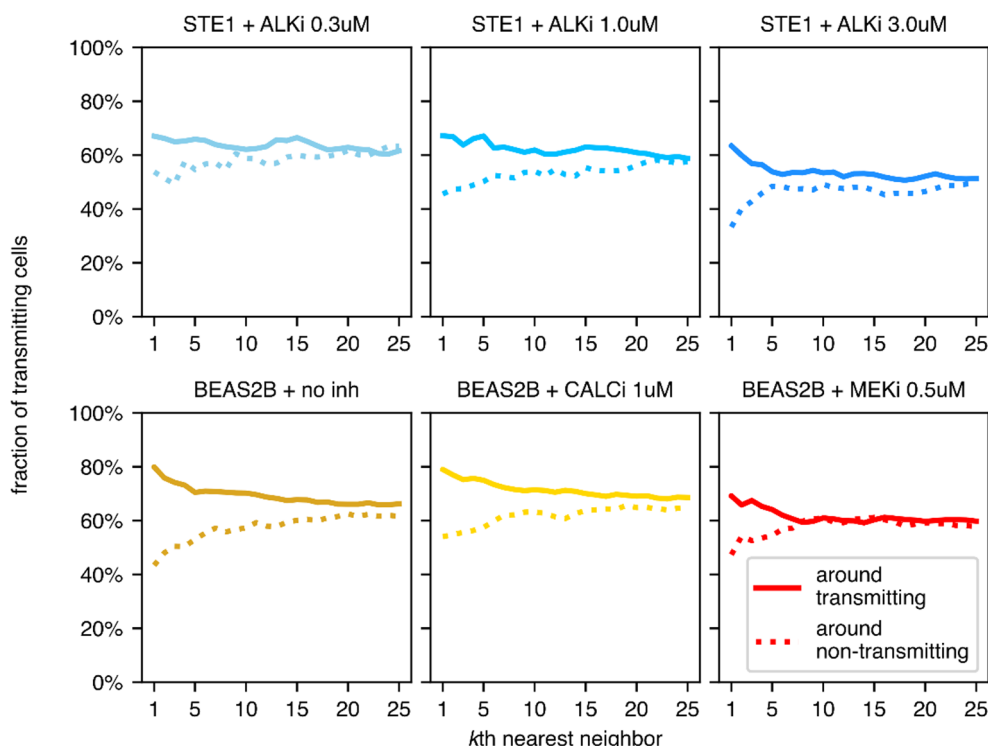


Fig. 4. Transmitting cells are spatially correlated. Graphs show the probability that the k th nearest neighbor of an information-transmitting (solid) or non-transmitting (dotted) cell transmits information.

Information-transmitting BEAS-2B cells tend to have both pathways functional

The fraction of transmitting BEAS-2B cells equals $65 \pm 5\%$ regardless of the application of MEKi or CALCi (Fig. 3C). This equality suggests that the transmitting subpopulation is common and in the ‘transmitting’ cells, both the RTK/calcineurin and the RTK/ERK channels are functional. To further investigate this conjecture, we trained separate neural classifiers to recognize stimulation pulses using either the calcineurin-mediated dip or the ERK-mediated peak. This was done by altering the ERK KTR trajectory slice provided to the network: for dip-based detection, the classifier had to decide on whether a pulse occurred at time t based on timepoints $[t, \dots, t+2 \text{ min}]$, while for peak-based detection, timepoints $[t+6 \text{ min}, \dots, t+12 \text{ min}]$ were provided. As expected, in ALKi-treated STE-1 almost no information could be retrieved by the dip-based detector (Fig. 5A), which implies that STE-1 cells did not transmit information via the calcineurin pathway. In contrast, BEAS-2B were able to respond with both pathways. As shown in Fig. 5B, there is a high correlation in information transmission between both pathways. A vast majority of cells transmit information through either both or none of the pathways with a small fraction of cells using a single channel only.

RTK/calcineurin pathway has shorter refractory time than RTK/ERK pathway

The average ERK-KTR trajectory after a light pulse depends on the cell line and the applied inhibitor (Fig. 6A). In addition, the ERK-dependent response amplitude depends on the pulse interval, increasing with the time that has passed since the previous light pulse (Fig. 6B). We observed the highest response amplitude in BEAS-2B cells without inhibitors or with CALCi. In STE-1 cells without ALKi the response was close to zero (Fig. 6A). With ALKi treatment, STE-1 cells began responding to light pulses, but the response amplitude remained consistently about 5 times smaller than in BEAS-2B cells treated with CALCi or left untreated (Fig. 6B). The response amplitude in BEAS-2B cells with MEKi was negative, as the RTK/calcineurin pathway leads to ERK-KTR dephosphorylation and translocation to, rather than from, the nucleus. Moreover, the amplitude was only weakly dependent on the time since the previous light pulse, suggesting that the RTK/calcineurin pathway has a very short refractory time. When the response amplitude was measured 2 min after the light pulse (Fig. 6C), the point at which the calcineurin-induced ERK-KTR dip was the strongest, we observed that both untreated BEAS-2B and BEAS-2B with MEKi responded to intervals as short as 5 min.

Consistent with earlier observations, the certainty of pulse detection by the MLP classifier (Fig. 6D) strongly depends on the response amplitude (Fig. 6B). The certainty can be measured as the mean logit Bayesian update to the predicted light pulse probability. The logit update is zero when the classifier cannot infer any additional information from the ERK-KTR trajectory, and thus predicts the probability of a light pulse equal to the prior probability. In contrast, the logit update approaches positive or negative infinity when the pulse is predicted or excluded with 100% certainty, respectively.

We found that in untreated BEAS-2B, pulses following a 5 min interval were typically undetectable. While the response amplitude (Fig. 6B) continued to increase with longer preceding intervals, detection credibility (Fig. 6D) plateaued at intervals of ~ 15 min, in agreement with previous work³⁵. Credibility reached half of its asymptotic value at an interval of 7–8 min, which we refer to as the effective refractory time. When calcineurin signaling was blocked with CALCi, detection of light pulses following intervals shorter than 20 min was worse than in untreated cells. This indicates that, in untreated cells, for short intervals, the network relies on both the ERK-mediated peak and the calcineurin-mediated dip to identify light pulses. The effective refractory time was increased to ~ 10 min. With only the RTK/calcineurin pathway active (BEAS-2B with MEKi), light pulse

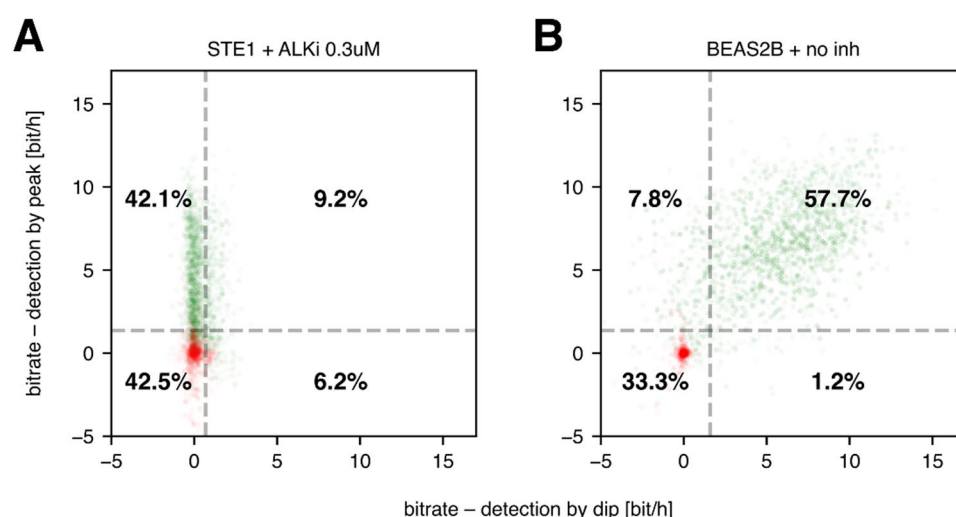


Fig. 5. Information transmission in the RTK/calcineurin and the RTK/ERK channels. Single cell bitrates determined based on calcineurin-mediated ‘dip’ or ERK-mediated ‘peak’ only. Color indicates transmitting (green) and non-transmitting (red) cells, determined as in Fig. 3. Dashed lines separate the transmitting and non-transmitting subpopulations determined based on the ‘dip’-only or ‘peak’-only detection. Numbers indicate fraction of cells in each quarter.

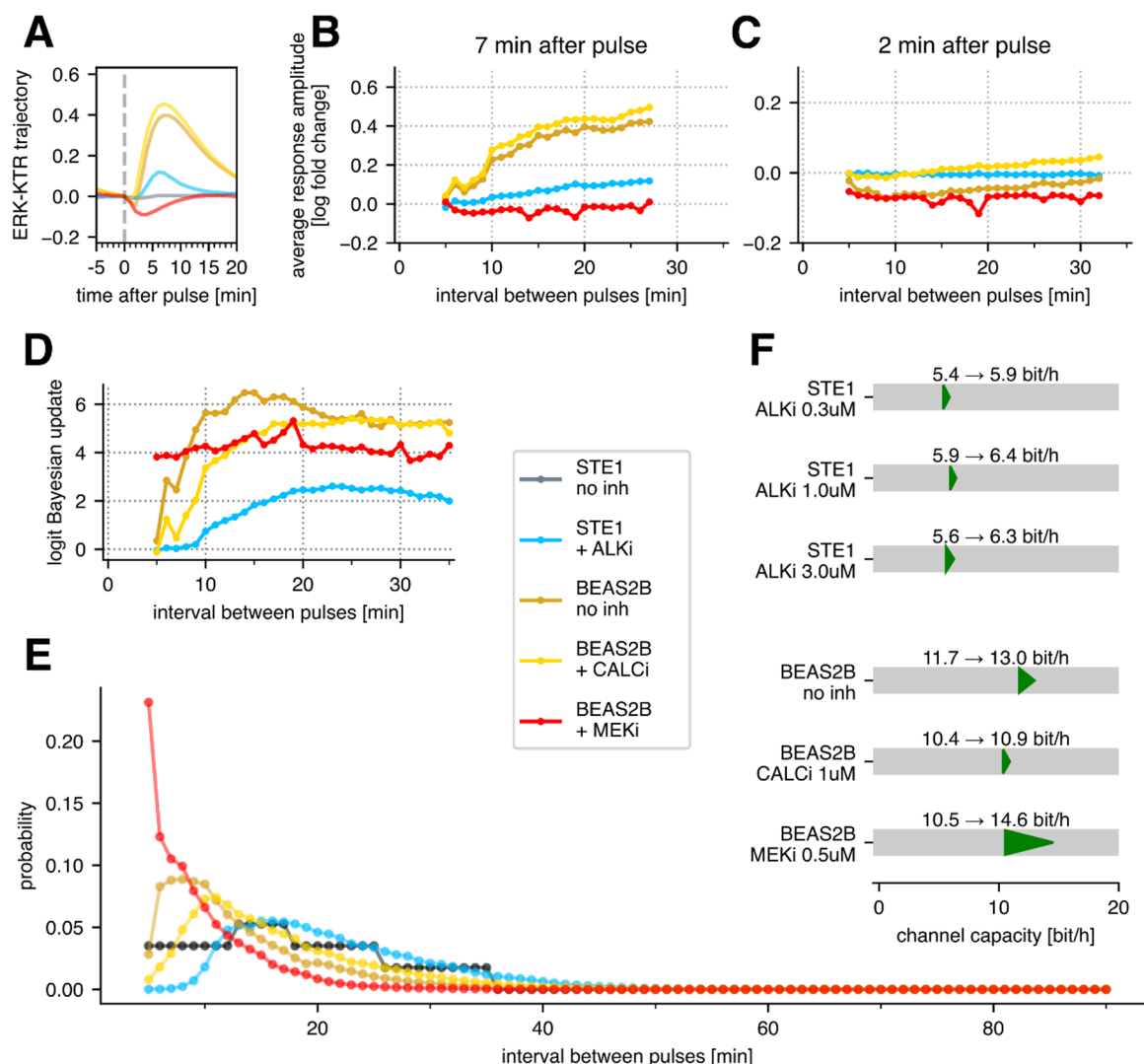


Fig. 6. In silico protocol optimization and channel capacity estimation. In panels A–E, colors correspond to different cell lines and conditions, as indicated in the common legend. All 0.3–3 μM ALKi concentrations were pooled. **(A)** Population-average ERK-KTR response to a light pulse preceded by a long interval (25 min). **(B)** Average response amplitude measured 7 min after pulse, as a function of the interval preceding the light pulse. **(C)** Same as B but measured 2 min after the pulse. **(D)** The MLP classifier’s logit Bayesian update to the prior pulse probability, as a function of the interval preceding the light pulse. **(E)** Optimized input interval distribution. The black line shows the interval distribution used in the experimental protocol. **(F)** Channel capacity (i.e., bitrate achievable with the optimized input interval distribution) in transmitting cells, compared to the experimentally determined bitrates reported in Fig. 3C. Data from all experimental replicates for a given cell line and condition were pooled and analyzed jointly. Panels D–F plotted based on transmitting cells only.

detection no longer depended on the interval within the tested range. This observation, consistent with Fig. 4C, indicates that the refractory time of the RTK/calceurin pathway is shorter than 5 min. This means that the measured refractory time of the RTK/ERK channel is intrinsic to the pathway and does not result from slow reporter dynamics.

In STE-1 cells with ALKi treatment, certainty reached saturation at ~20 min intervals, as in BEAS-2B with CALCi. However, even at saturation, the neural network’s certainty remained markedly lower in STE-1 cells compared to BEAS-2B. Detection of light pulses became possible after intervals longer than 9 min, and crossed the half-asymptotic threshold at ~13 min, implying an effective refractory time ~3 min longer than in BEAS-2B with CALCi. The longer refractory time in STE-1 might suggest a discrepancy in a negative feedback mechanism between the two cell lines. However, because the response in STE-1 was weaker yet rose with similar timing as in BEAS-2B with CALCi (Fig. 6B), we suspect that the primary cause of the longer effective refractory time is an insufficient signal-to-noise ratio for short intervals in treated STE-1.

In silico protocol optimization indicates that the RTK/ERK channel capacity is 5–15% higher than the experimentally estimated bitrate

Throughout our work, we assume that the length of intervals between subsequent pulses constitutes the ‘alphabet’ for information encoding; particular interval lengths (‘letters’) are independent, and thus, the bitrate depends only on the frequency of particular intervals. Thus far, we have estimated the bitrate assuming a fixed distribution of intervals shown in Fig. 1J. Although the interval distribution was chosen on the basis of preliminary data to yield a high bitrate for the RTK/ERK channel, it does not have to be optimal for both cell lines and, more importantly, for the RTK/calcieneurin pathway. In contrast to our previous work²⁵, the bitrate estimation algorithm employed in this study allowed us to use existing experimental data to make bitrate predictions for protocols that have not been experimentally tested (see Methods). Since the channel capacity is the maximum bitrate that can be achieved by optimizing the encoding protocol, this allowed us to estimate the channel capacity of the investigated pathways in silico without the need to conduct multiple experimental trials. Specifically, for each cell line and inhibitor (all STE-1 experiments with ALKi were pooled), we searched for the optimal input protocol by numerical optimization of the input interval distribution using gradient descent.

We optimized over protocols with interval lengths from 5 min to 90 min. To allow for intervals longer than 35 min (the longest interval in our dataset), we extrapolated cell responses based on the 35-min interval, which was justified by the detection certainty reaching saturation after approximately 20 min (Fig. 6D; see Methods for details). In most conditions, the cells did not respond to pulses following a 5-min interval; thus, we assumed that shorter intervals, unavailable in our experimental dataset, might be safely excluded from the optimized protocols. The only exception was BEAS-2B with MEKi, for which the data presented in Fig. 6B–D suggest that pulses following intervals shorter than 5 min may be detectable; consequently, the true channel capacity of the RTK/calcieneurin pathway is likely higher than estimated here.

Most of the optimal interval distributions (Fig. 6E) follow a qualitatively similar pattern. Intervals below the detectability threshold are not sent; the interval probability rises steeply following the increase in pulse amplitude and detection certainty (Fig. 6B–D). Once the detection certainty has reached a plateau, the interval probability starts to decrease because the usage of long intervals requires more time to relay the same amount of information. The optimal trade-off between the sent information content and time budget per pulse is granted by the exponentially decreasing distribution, which we observe in all optimal protocols. In most conditions, the change in interval distribution due to optimization increased the bitrate by 5–15% (Fig. 6F). The only exception was BEAS-2B with MEKi, which experienced nearly 40% gain in the bitrate. In this case, the optimal distribution was significantly shifted toward shorter intervals, which did not affect pulse detection certainty (roughly independent of interval length; Fig. 6D) but increased the input information rate.

Overall, we found that ALKi treatment of STE-1 cells restored the RTK/ERK channel capacity to approximately 6.0–6.5 bit/h in the transmitting subpopulation. However, this value remains below the RTK/ERK channel capacity of approximately 11 bit/h estimated for BEAS-2B. The RTK/calcieneurin channel in BEAS-2B cells was found to have an even higher capacity of nearly 15 bit/h (which, as mentioned before, is likely underestimated). Surprisingly, this capacity exceeds the capacity of BEAS-2B cells when both the RTK/calcieneurin and the RTK/ERK pathways are unblocked (13 bit/h). This effect is probably caused by signal interference: the rise of the ERK-mediated peak counteracts the calcieneurin-mediated dip (Fig. 6A) and thus hampers the dip-based detection.

Discussion

The rate at which information is transmitted by signaling channels constrains the complexity of processes that can be regulated within a given time. High bitrates are expected for signaling pathways that regulate cellular responses to rapidly varying extracellular cues. The RTK/ERK pathway, which governs proliferation, differentiation, apoptosis, and motility, is one such pathway, and due to the nature of these processes, its malfunction is frequently associated with cancer. We note that higher bitrates are not necessarily optimal. For example, an overly-responsive cell could proliferate in response to otherwise subthreshold stimuli, leading to pathological cell growth. Rather we expect that cellular information transmission is tuned to respond to the signals—and filter the noise—of the particular tissue environment, and that disease states or molecular interventions can meaningfully alter this perceptive landscape.

In this study, using pulsatile optoFGFR stimulation, we showed that the presence of the EML4-ALK oncogene in STE-1 cells suppressed RTK/ERK signaling, reducing the pathway’s bitrate to nearly zero. However, treatment with an ALK inhibitor restored information transmission. With the inhibitor, the transmitting subpopulation of cells—comprising approximately 50–60% of all STE-1 cells—achieved an average bitrate of ~5.5 bit/h. This rate was lower than that of the RTK/ERK pathway in noncancerous BEAS-2B cells, where the transmitting subpopulation of approximately 70% reached an average bitrate of ~10.4 bit/h. The fraction of transmitting cells varied substantially across experimental replicates, which affected the average bitrate calculated across the entire population. By contrast, the average bitrate within the transmitting subpopulation (reported above) showed much lower variability and therefore can be considered a reliable metric. The reasons why the remaining cells failed to transmit information remain unclear. The immediate cause was typically a lack of response to stimulation, but we failed to attribute it to optoFGFR expression (cells with low optoFGFR expression were excluded), ERK-KTR level, nucleus size, or other factors like position within the colony or local cell density.

The lower bitrate in ALKi-treated STE-1 cells (with respect to BEAS-2B) results primarily from a lower response amplitude (higher noise to signal ratio) and a longer refractory period, leading to reduced accuracy of reconstruction. The strong correlation between the average ERK-KTR response amplitude and bitrate measured in single cells suggests that the ~5-fold weaker response strength in treated STE-1 cells may be the underlying cause of the longer measured refractory period.

In BEAS-2B cells (as in MCF-10 A cells²⁵), two pathways activated by optoFGFR transmit opposing signals to ERK-KTR: the RTK/ERK pathway and the RTK/calcieneurin pathway. The reporter is sensitive to

calcineurin signaling because it contains an ERK docking site from the transcription factor ELK-1, which is also a substrate for dephosphorylation by calcineurin^{30,31}. The RTK/calcineurin pathway thus induces rapid ERK-KTR dephosphorylation and nuclear translocation, starting 2 min after light pulse stimulation (of note, this rapid nuclear ERK-KTR translocation was not observed in STE-1). In contrast, the RTK/ERK pathway leads to ERK-KTR phosphorylation and cytoplasmic translocation, peaking approximately 7 min after the light pulse. Consequently, in BEAS-2B cells without inhibitors, ERK-KTR trajectories after a light pulse display a small ‘dip’ followed by a more pronounced ‘peak.’ By applying either a calcineurin or a MEK inhibitor, we investigated these pathways in isolation and estimated that each transmits information at a similar rate of 10–11 bit/h (again, restricting the estimation to the transmitting subpopulations). These rates are only slightly lower than the bitrate achieved without inhibitors. Our data additionally indicate that, in response to RTK stimulation, ELK-1 might undergo rapid dephosphorylation followed by phosphorylation; the biological meaning of such regulation remains to be further studied.

While bitrate is a quantity dependent on the stimulation protocol, channel capacity, i.e. bitrate in an optimal protocol, is a universal measure. The pulsatile stimulation protocol used in the study was designed to maximize the bitrate of the RTK/ERK pathway on the basis of preliminary experiments and our previous study on MCF-10 A cells²⁵. It balances high information input, achieved with short intervals between stimulation pulses, against signal transmission accuracy, which deteriorates when intervals become as short as the refractory time. However, since the refractory time varies between cell lines and signaling pathways, the protocol could not be optimal simultaneously for all conditions. To address this, we developed an approach for optimizing stimulation protocols and estimating channel capacities from a single suboptimal experiment. This method works best when all intervals expected in the optimal protocol are present in the experimental protocol. Otherwise, responses to the missing intervals must be extrapolated. While the limited experiment time forced us to extrapolate data for intervals above 35 min, our data suggest that this extrapolation is generally justified. However, lack of intervals shorter than 5 min in the experimental protocol and inability to extrapolate them probably lead to an underestimation of the channel capacity of the RTK/calcineurin pathway in BEAS-2B cells. Although the optimized protocol differs from the experimental one, the estimated RTK/ERK channel capacities in BEAS-2B cells and STE-1 cells (with an ALK inhibitor) are only 5–15% higher than the experimentally measured bitrates, and equal 13 bit/h and ~6.5 bit/h, respectively. This shows that the near-optimal bitrate can be achieved by a relatively broad family of protocols. For the RTK/calcineurin pathway, the bitrate increase is much greater – approximately 40%. The high RTK/calcineurin channel capacity (~15 bit/h) results from its short refractory time, allowing transmission of sequences with short intervals. As mentioned, this estimate should be considered a lower bound, as even higher bitrates might be achieved with stimulation intervals shorter than 5 min. Indeed, calcium signaling plays a role in regulating rapid cellular processes, such as actin remodeling during cell movement or fertilization^{32–34}.

We emphasize that all bitrates and channel capacities reported in this paper quantify information transmission from optoFGFR to ERK-KTR and thus represent lower bounds for the information reaching the upstream proteins (ERK and calcineurin). Direct measurements of ERK or calcineurin activity could yield higher values, though such measurements are currently not possible for time-resolved measurements in single cells. The measured refractory time for the RTK/ERK pathway is much longer than that of the RTK/calcineurin pathway, and therefore cannot be limited by slow ERK-KTR dynamics. Because ERK-KTR was designed based on an ERK-dependent transcription factor (ELK-1), the reported values can also be interpreted as an estimate of information available to ERK-dependent transcription factors. Some information may have been lost during processing by the neural classifier; however, the effect is likely negligible, as altering the network architecture did not improve prediction accuracy. Bitrates may also be underestimated due to the finite temporal resolution of stimulation and measurement. Nevertheless, as shown in Supplementary Fig. S5, even with the current 1-minute resolution, the network could not reliably distinguish the absence of a pulse at the time point immediately preceding stimulation. Therefore, encoding protocols with higher than 1-min temporal resolution is unlikely to increase the bitrate substantially.

In summary, we developed a machine learning-based method to analyze information flow in signaling channels and its changes as a function of an oncogenic state. We found high information capacities that confirm that ERK and calcineurin pathways can efficiently transmit signals from receptors to effector proteins. Low bitrate in EML4-ALK-positive cells highlights the impaired transmission in cancer cells, which can be at least partially restored by drug treatment. Further studies will address whether such high bitrates are fully utilized in physiological responses, whether their suppression plays a role in cancer development, and whether drug-induced relief of suppression plays an important role during therapy, for example, in drug resistance.

Methods

Materials

Cell lines

BEAS-2B cells were purchased from ATCC. STE-1 cells were a gift from Trever Bivona lab, UCSF.

Inhibitors

Crizotinib (Sigma-Aldrich, PZ0191) was used as an ALK inhibitor (ALKi).

Trametinib (Selleckchem, GSK1120212) was used as a MEK inhibitor (MEKi).

Cyclosporin A (Thermo Fisher Scientific, AAJ6319103) was used as a calcineurin inhibitor (CALCi).

Plasmid expression

The optoFGFR-encoding plasmid CLPIT Myr-mEGFP-FGFR(ICD)-Cry2 was cloned by substitution of mCherry with mEGFP in CLPIT Myr-mCherry-FGFR(ICD)-Cry2¹².

The cells were infected sequentially with pLentiPGK DEST-H2B-iRFP670 (Addgene #90237) for nuclear imaging, pHR ErkKTR-mRuby2 or pHR ErkKTR-mCherry (STE-1 or BEAS-2B, respectively) for ERK activity measurement and CLPIT Myr-mEGFP-FGFR(ICD)-Cry2 for stimulation.

Infected cells were sorted twice to enrich for high expression of all 3 markers.

Experimental procedure

Cell culture

The cells were cultured at 37 °C and 5% CO₂ in RPMI-1640 growth medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). For the experiments, cells (STE-1, 1.5×10^4 ; BEAS-2B, 5×10^3) were seeded 48 h before the experiment in 96-well plates (Cellvis) coated with fibronectin (MilliporeSigma, FC01010MG) diluted to 10 µg/mL in PBS. The media were replaced 16 h before the experiment with RPMI-1640 w/o phenol red, either serum-free or containing 4% FBS (for BEAS-2B or STE-1, respectively). Upon the initiation of the experiment, an equal volume of serum-free medium with supplements or DMSO was added to reach the indicated concentrations.

Imaging

Live-cell imaging was performed using a Nikon Ti2-E microscope equipped with a Yokagawa CSU-W1 spinning disk, 405/488/561/640 nm laser lines, an sCMOS camera (Photometrics), and a motorized stage. The cells were maintained at 37 °C and 5% CO₂ in an environmental chamber (Okolabs). The cells were imaged every 60 s for 90 min without optoFGFR stimulation, followed by the stimulation protocol described in the next subsection. The 561 nm line was used for ERK-KTR-mRuby2 and ERK-KTR-mCherry imaging (80% laser power; 150 ms), and the 640 nm line was used for H2B-iRFP imaging (80% laser power; 200 ms). OptoFGFR was stimulated and imaged with 488 nm (100% laser power, i.e., 1.15 W/cm²; 100 ms) at the indicated time points.

Stimulation sequence

In all the experiments, the cells were stimulated with an identical sequence of light pulses (Supplementary Table S1). The sequence was constructed as follows. First, on the basis of preliminary data and earlier results²⁵, we decided that the intervals between subsequent light pulses should approximately follow a Gamma distribution with shape $\alpha = 4$ and scale $\theta = 5$ min. This choice was expected to be near-optimal in terms of bitrate and broad enough to probe various interval lengths. The actual number of intervals of a particular length in the sequence (Fig. 1I) was chosen to best reflect this distribution given the time budget (~17 h). Minor adjustments were made to ensure that each short interval length occurred at least twice. The intervals were ordered in such a way that the occurrences of a particular interval length were preceded by intervals maximally representative of the assumed distribution. For example, there were three intervals of length 13 min in the sequence; they were preceded by intervals of lengths 7, 17, and 28 min, which were close to the 1st, 2nd, and 3rd quartiles of the assumed distribution, respectively. This was important for a fair comparison between interval lengths (Fig. 6) because a pulse following a short interval evokes a stronger response if the interval before it was also short; consequently, if all intervals of a particular length were preceded by short intervals, the detectability of this interval length would be overestimated.

Image processing

Cell tracking

The cell nuclei were detected based on H2B-iRFP fluorescence and tracked using our in-house software ShuttleTracker. Tracks shorter than 3 h were removed from further analysis.

Track standardization

We estimated the background intensity in each channel as the mode of the pixel intensity (a common value was chosen across all frames and replicates with equal microscope settings) and subtracted it from the image. For each cell in a frame, we measured the average nuclear intensity in the ERK-KTR channel and normalized it with the average intensity over the whole frame. These two steps were necessary to eliminate global changes in mRuby2 fluorescence arising in response to the 488 nm light used for optoFGFR stimulation and to compensate for illumination differences across frames and replicates. The time course of the negative logarithm of the obtained value is referred to as a normalized *ERK-KTR trajectory*. We used this value instead of the typically used cytoplasmic-to-nuclear ratio to avoid noise associated with inaccurate cytoplasm detection. For a clearer graphical presentation in Fig. 2A, the single-cell ERK-KTR trajectories were normalized by subtracting the average value in each track.

Preselection of optoFGFR-expressing cells

We sorted the tracked nuclei in each replicate based on the mean optoFGFR fluorescence intensity at the time points at which the optoFGFR was stimulated and discarded tracks outside the range $[\mu, \mu + 3\sigma]$ (BEAS-2B) or $[\mu - 0.5\sigma, \mu + 2\sigma]$ (STE-1), where μ and σ denote the track-length-weighted average and standard deviation across all tracks in the particular replicate (see Supplementary Fig. S1).

Response amplitude and responsiveness

For each track and light pulse, we computed the *response amplitude* as the log-ratio of the ERK-KTR trajectory 7 min after the pulse and at the pulse. To account for the influence of the previous pulse, we normalized it by subtracting the average log-ratio of the ERK-KTR trajectory ($L + 7$ min) and L after the pulse, where L is the interval between this pulse and the previous pulse. The average was taken across all tracks and all pulses followed by at least $(L + 7$ min)-long intervals (Supplementary Fig. S2).

We also computed the cell's *responsiveness* as the standard deviation across all time points (not just time points with pulses) of the log fold change in translocation over 7 min. This measure was independent of pulse timing and, as such, could be used as a hint in automated pulse detection, as described below.

Bitrate computation

Derivation of the bitrate lower bound

We consider random sequences of light pulses that may occur in whole minutes. These sequences are encoded as $X = X_1, \dots, X_N$, where $X_k = 1$ if there was a pulse sent at the k th minute and $X_k = 0$ otherwise. We note that X_k need not be independent. For each cell, we denote the sequence of responses (ERK-KTR trajectories) as $Y = Y_1, \dots, Y_N$. The mutual information between these two sequences can be computed as:

$$\begin{aligned} I(X; Y) &= H(X) - H(X|Y) \\ &= \sum_k [H(X_k | X_{1 \dots k-1}) - H(X_k | X_{1 \dots k-1}, Y)] \\ &= - \sum_k [\mathbb{E} \log p(X_k | X_{1 \dots k-1}) - \mathbb{E} \log p(X_k | X_{1 \dots k-1}, Y)] \\ &= - \sum_k \mathbb{E} [\log p(X_k | X_{1 \dots k-1}) - \log p(X_k | X_{1 \dots k-1}, Y)] \end{aligned} \quad (1)$$

For independent X_k , $H(X_k | X_{1 \dots k-1}) = H(X_k)$. In this case, the intervals between pulses follow a geometric distribution. However, we consider arbitrary interval distributions, and thus $H(X_k | X_{1 \dots k-1}) \leq H(X_k)$.

For a sufficiently large number of time points, N , the bitrate (or transmitted information per time point), $b(X; Y)$, can be estimated as:

$$\begin{aligned} b(X; Y) &= I(X; Y) / N \\ &= - \frac{1}{N} \sum_k \mathbb{E} [\log p(X_k | X_{1 \dots k-1}) - \log p(X_k | X_{1 \dots k-1}, Y)] \\ &= - \mathbb{E} [\log p(X_k | X_{1 \dots k-1}) - \log p(X_k | X_{1 \dots k-1}, Y)] \end{aligned} \quad (2)$$

where in the last equation, the expected value is also calculated over all time points $k = 1, \dots, N$. Thus, $b(X; Y)$ is equal to the expected difference between the surprisal of X_k conditioned on all previous pulses, $-\log p(X_k | X_{1 \dots k-1})$, and the surprisal of X_k conditioned on all previous pulses and additionally the full response Y , $-\log p(X_k | X_{1 \dots k-1}, Y)$. This can be thought of as the expected reduction in surprisal from learning Y .

To estimate $b(X; Y)$, we first note that the probability $p(X_k | X_{1 \dots k-1})$ depends only on the assumed encoding protocol. We restrict our analysis to protocols for which the probability of a pulse at time point k depends only on the time that elapsed since the previous pulse, $Last_k$ ($Last_k = l$ if and only if $X_{k-l} = 1$ and $X_{k'} = 0$ for all $k' \in \{k-l+1, \dots, k-1\}$). Thus:

$$p(X_k | X_{1 \dots k-1}) = p(X_k | Last_k) \quad (3)$$

The posterior probability $p(X_k | X_{1 \dots k-1}, Y)$ cannot be directly computed. Instead, we estimate it by training a neural network p_θ (with weights θ) to predict X_k . In principle, the neural network could use the complete stimulation history $X_{1 \dots k-1}$ and the full response Y to predict X_k . However, we simplify this by reducing $X_{1 \dots k-1}$ to $Last_k$, and Y to a slice $Y_{k \dots k+r}$ (of length $r+1=7$, starting at time point k) and a scalar measure of cell responsiveness $S = \text{Std}(Y_k - Y_{k+7})$. This reduction is necessary, as the network is trained and tested on the same sequence of pulses; thus, the stimulation history $X_{1 \dots k-1}$, as well as the full response trajectory Y , can be used to infer the timepoint in the experiment (k) and consequently reveal X_k . By providing the network with $Last_k$ instead of $X_{1 \dots k-1}$ and the slice $Y_{k \dots k+r}$ instead of Y , we prevent this leak. In summary, we approximate the posterior probability as:

$$\begin{aligned} p(X_k | X_{1 \dots k-1}, Y) \\ \approx p(X_k | Last_k, Y_{k \dots k+r}, S) \\ \approx p_\theta(X_k | Last_k, Y_{k \dots k+r}, S) \end{aligned} \quad (4)$$

This approximation results in an upper bound on the conditional entropy:

$$\begin{aligned} H(X_k | X_{1 \dots k-1}, Y) &\leq H(X_k | Last_k, Y_{k \dots k+r}, S) \\ &\leq -\mathbb{E} [\log p_\theta(X_k | Last_k, Y_{k \dots k+r}, S)] \end{aligned} \quad (5)$$

where the expectation is still computed over the true distribution of X, Y , and time points k . The first inequality follows from the data processing inequality, and the second reflects the fact that the cross-entropy between p and p_θ is an upper bound on the entropy of p . Thus, by substituting (5) back into formula (2), we obtain a lower bound on the bitrate:

$$b(X; Y) \geq -\mathbb{E} [\log p(X_k | Last_k) - \log p_\theta(X_k | Last_k, Y_{k \dots k+r}, S)] \quad (6)$$

The bound is tight, assuming that the network correctly predicts the true posterior probability (both approximations in Eq. (4) are equalities). We estimate the bitrate by averaging over all cells j and timepoints k available in our dataset:

$$b = -\frac{1}{D} \sum_{j,k} [\log p(x_k | last_k) - \log p_\theta(x_k | last_k, y_{k \dots k+r}^j, s^j)] \quad (7)$$

where D is the number of data points (j, k) , and small letter symbols x_k and $last_k$ denote values from distributions X_k and $Last_k$.

Single-cell bitrates

We define the bitrate of a given cell track j as:

$$\begin{aligned} b_j &= -\mathbb{E}_k [\log p_\theta(X_k | Last_k) - \log p_\theta(X_k | Last_k, Y_{k \dots k+r}, S)] \\ &= -\frac{1}{w_j} \sum_k [\log p(x_k | last_k) - \log p_\theta(x_k | last_k, y_{k \dots k+r}^j, s^j)] \end{aligned} \quad (8)$$

where w_j is the number of timepoints available for cell track j . Note that since we use a model trained on all tracks, if a cell responds aberrantly, it can cause the model to make worse predictions than the prior, resulting in a negative bitrate of that track. The overall bitrate can be expressed as an average over the individual cell track bitrates (weighted by cell track length):

$$\begin{aligned} b &= -\frac{1}{D} \sum_{j,k} [\log p(x_k | last_k) - \log p_\theta(x_k | last_k, y_{k \dots k+r}^j, s^j)] \\ &= -\frac{1}{D} \sum_j w_j \left(\frac{1}{w_j} \sum_k [\log p(x_k | last_k) - \log p_\theta(x_k | last_k, y_{k \dots k+r}^j, s^j)] \right) \\ &= \frac{1}{D} \sum_j w_j b_j = \frac{\sum_j w_j b_j}{\sum_j w_j} \end{aligned} \quad (9)$$

Since w_j is the number of timepoints available for cell j , $D = \sum_j w_j$.

Fraction of transmitting cells

The fraction of transmitting cells is determined by sorting the single-cell bitrates in ascending order and taking the longest prefix that averages (weighted by cell track length) to a negative value. The cells in the prefix are called non-transmitting, whereas the rest constitute the transmitting subpopulation.

Network training procedure

Data sampling

The network is trained to minimize the cross-entropy loss function using stochastic gradient descent:

$$\mathcal{L} = -\mathbb{E} \log p_\theta(X_k | Last_k, Y_{k \dots k+r}, S) \quad (10)$$

At each step, the expected value is computed by averaging over a mini-batch of 10^4 data points $(x_k, last_k, y_{k \dots k+r}^j, s^j)$ randomly drawn from the dataset. In general, the network predictions p_θ depend on the assumed input protocol (interval distribution). Although throughout the paper, we use a network trained according to the experimentally tested interval distribution, we designed the sampling procedure to allow for training and evaluation using an arbitrary protocol. We group all the data points by $interval_k$ (the length of the interval containing the data point) and $last_k$ (the time elapsed since the previous pulse). We first sample pairs $(interval_k, last_k)$ according to their distribution in the assumed protocol, and then randomly retrieve data points from the corresponding groups in the experimental dataset to form mini-batches.

Procedure:

1. Group dataset by $(interval_k, last_k)$.
2. Sample pairs of $(interval_k, last_k)$ from the joint distribution of the assumed protocol.
3. Select random samples from the dataset for each $(interval_k, last_k)$ pair.

Multiple experiments

Throughout the paper, we use a single network trained on all the experiments. We allow the network to adapt to different experimental settings by supplying additional inputs:

$$\mathcal{L} = -\mathbb{E} \log p_\theta(X_k | Last_k, Y_{k \dots k+r}, S, CellLine, Inhibitor) \quad (11)$$

where *CellLine* is 0 for STE1 and 1 for BEAS-2B, and *Inhibitor* is a triplet of real numbers providing concentrations of 3 inhibitors {*ALKi*, *MEKi*, *CALCi*}. We then performed leave-one-out cross-validation on the experimental replicates (see Supplementary Fig. S3). Since the change in bitrate estimate after exclusion of any single replicate is negligible, we concluded that the network does not overfit to individual replicates and that we may use a single network trained on all available data.

Network architecture

The network inputs are $\log(last_k)$, $y_{k \dots k+r}^j$, s^j , *cellLine*, and *inhibitor* (3-dim). The trajectory slice $y_{k \dots k+r}^j$ is replaced with discrete differences $\Delta y_{k+1 \dots k+r}^j$ where $\Delta y_{k'}^j = y_{k'}^j - y_{k'-1}^j$, to abstract from the base level of ERK-KTR expression in individual cells. We chose to use the slice of $r=6$ discrete differences, as its further extension provides no substantial increase in bitrate (see Supplementary Fig. S4). To detect pulses based on the 'dip' or 'peak'-only (Fig. 5), we use, respectively, slices $y_{k \dots k+2}^j$ and $y_{k+6 \dots k+12}^j$. All the inputs are concatenated, resulting in a feature vector of length $(r+6)$. The features are standardized across the dataset to have zero mean and a standard deviation of $\sigma = 1$, and passed to a three-layer perceptron (MLP) with hidden layer sizes of (40, 20) and leaky ReLU activations. The MLP computes a logit (Bayesian) update u_{Bayes} to the prior probability $p(X_k = 1 | Last_k)$:

$$u_{Bayes} = MLP(\log last_k, \Delta y_{k+1 \dots k+r}^j, s^j, cellLine, inhibitor) \quad (12)$$

This update is then used to compute the posterior (logit) probability of $X_k = 1$:

$$\text{logit } p_{\theta}(X_k = 1 | last_k, y_{k \dots k+r}^j, s^j, cellLine, inhibitor) = u_{Bayes} + \text{logit } p(X_k = 1 | last_k) \quad (13)$$

This approach allows the network to generalize more effectively across protocols with different priors $p(X_k = 1 | Last_k)$, simplifying protocol optimization.

Supplementary Fig. S5 illustrates the mean updates u_{Bayes} generated by the MLP, grouped by $(interval_k, last_k)$ for STE-1 with ALKi. A positive value indicates that the network consistently predicts a higher pulse probability than the prior probability—this is the case along the diagonal ($interval_k = last_k$), corresponding to timepoints with actual pulses ($X_k = 1$). A value below zero indicates that the network predicts a lower pulse probability than the prior. A value close to zero implies that the network cannot improve upon the prior probability. As a result, no information is gained. This behavior is observed for $last_k \leq 10$, where cells have not yet recovered from a previous pulse and cannot respond to a new pulse (see also Fig. 6B). Consequently, their ERK-KTR trajectory carries no information about a potential new pulse. For $last_k > 10$, off-diagonal terms (where $X_k = 0$) are generally negative. For such $last_k$, a pulse would evoke a noticeable ERK-KTR trajectory change; thus, the absence of such a change leads the network to infer that no pulse has occurred and predict a probability lower than the prior probability. Importantly, these negative predictions also contribute to the total information transmitted and thus the bitrate. Finally, we note that entries immediately next to the diagonal are also close to zero. This suggests that the network sees a response to the pulse but is not perfectly sure of the exact timing of the pulse.

Protocol optimization

To optimize the protocol, we want to directly maximize the bitrate estimate:

$$\mathcal{L} = -\mathbb{E}[\log p(X_k | Last_k) - \log p_{\theta}(X_k | Last_k, Y_{k \dots k+r}, S)] \quad (14)$$

This introduces several challenges. First, during training, the protocol's interval probabilities are optimized via gradient descent. However, the expected value is computed by sampling according to the protocol; thus, we need to compute a derivative of the form $\nabla_{\phi} \mathbb{E}_{z \sim Z(\phi)} f(z)$ for some distribution Z parameterized by ϕ . This is accomplished by rewriting the expected value using a frozen distribution with importance weights:

$$\mathbb{E}_{z \sim Z(\phi)} f(z) = \left[\mathbb{E}_{z \sim Z(\phi')} f(z) \frac{p_{\phi}(z)}{p_{\phi'}(z)} \right] \Big|_{\phi'=\phi} \quad (15)$$

Since the sampled distribution no longer depends on ϕ , the gradient ∇_{ϕ} may be calculated as follows:

$$\begin{aligned} \nabla_{\phi} \left[\mathbb{E}_{z \sim Z(\phi')} f(z) \frac{p_{\phi}(z)}{p_{\phi'}(z)} \right] \Big|_{\phi'=\phi} \\ = \left[\mathbb{E}_{z \sim Z(\phi')} \nabla_{\phi} \left(f(z) \frac{p_{\phi}(z)}{p_{\phi'}(z)} \right) \right] \Big|_{\phi'=\phi} \end{aligned} \quad (16)$$

Although the importance weights $p_{\phi}(z)/p_{\phi'}(z)$ are equal to 1, their derivatives with respect to ϕ in general differ from zero and allow us to compute the gradient. In our case, we sample from a frozen protocol and update the protocol after each gradient step is calculated.

Secondly, the optimal protocol should assign nonzero probabilities to intervals longer than 35 min, the longest interval used in our experiments. Since we observe that responses for intervals longer than 30 min are similar (and strongest), we handle longer intervals by imputing them using the available 35-minute interval from our experiments. Specifically, when a trajectory slice for a pair $(interval_k, last_k)$ with $interval_k > 35$ min is

requested, we return a slice for an imputed pair $(interval_k^*, last_k^*)$ instead, where $interval_k^* = 35$ min and $last_k^*$ is determined as follows:

- If $last_k < 10$ min: $last_k^* = last_k$
- If $interval_k - last_k < 10$ min: $last_k^* = 35$ min $- (interval_k - last_k)$.
- Otherwise:

$$last_k^* = 10 \text{ min} + (last_k - 10 \text{ min}) \times \frac{35 \text{ min} - 20 \text{ min}}{interval_k - 20 \text{ min}} \quad (17)$$

In this way, we keep the time since the last pulse and the time to the next pulse fixed if this time is shorter than 10 min. A graphical illustration of imputation for $interval_k = 60$ min is shown in Supplementary Fig. S6. Note that while $y_{k \dots k+r}$ is imputed, the network is provided with true $last_k$.

Furthermore, selection of the transmitting subpopulation technically depends on single-cell bitrates, and thus also depends on the protocol. We perform protocol optimization on the transmitting subpopulation determined using the experimental protocol.

Finally, the optimized protocol can exhibit large jumps in probabilities of specific intervals owing to experimental noise and the order in which intervals were tested in the experiment. To address this, we introduce a regularization term in the loss function to promote smoother protocols:

$$\mathcal{L}_{reg} = \mathcal{L} - \alpha \|\Delta\Delta \text{ProtocolLogProbabilities}\|_2^2 \quad (18)$$

where $\Delta\Delta\text{ProtocolLogProbabilities}$ represents the second-order differences in the interval log-probabilities, and $\|\cdot\|_2^2$ is the mean of squares. We found that setting $\alpha = 0.003$ yields noticeably smoother protocols with minimal impact on the bitrate (see Fig. 6E and Supplementary Fig. S7).

Data availability

Data used in the paper (time-lapse microscopy quantifications) are deposited on Zenodo (<https://doi.org/10.5281/zenodo.15282876>). Raw images can be obtained from Tomasz Lipniacki (tlipnia@ippt.pan.pl). Source code is available on GitHub (<https://github.com/pawelnalecz/pathway-bitrate>). Cell lines and plasmids generated for this study are available from Lukasz J. Bugaj (bugaj@seas.upenn.edu).

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Author contributions

P.N.-J., L.R., F.G., M.K., L.J.B., and T.L. designed the project. S.L. and L.R. cloned the expression vectors. L.R. developed the cell lines and performed the experiments. P.N.-J. and M.K. performed image analysis, P.N.-J., and F.G. developed the mathematical methods and analyzed the data. All authors contributed to writing the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-025-23075-y>.

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Supplementary Materials

Quantifying cancer- and drug-induced changes in Shannon information capacity of RTK signaling

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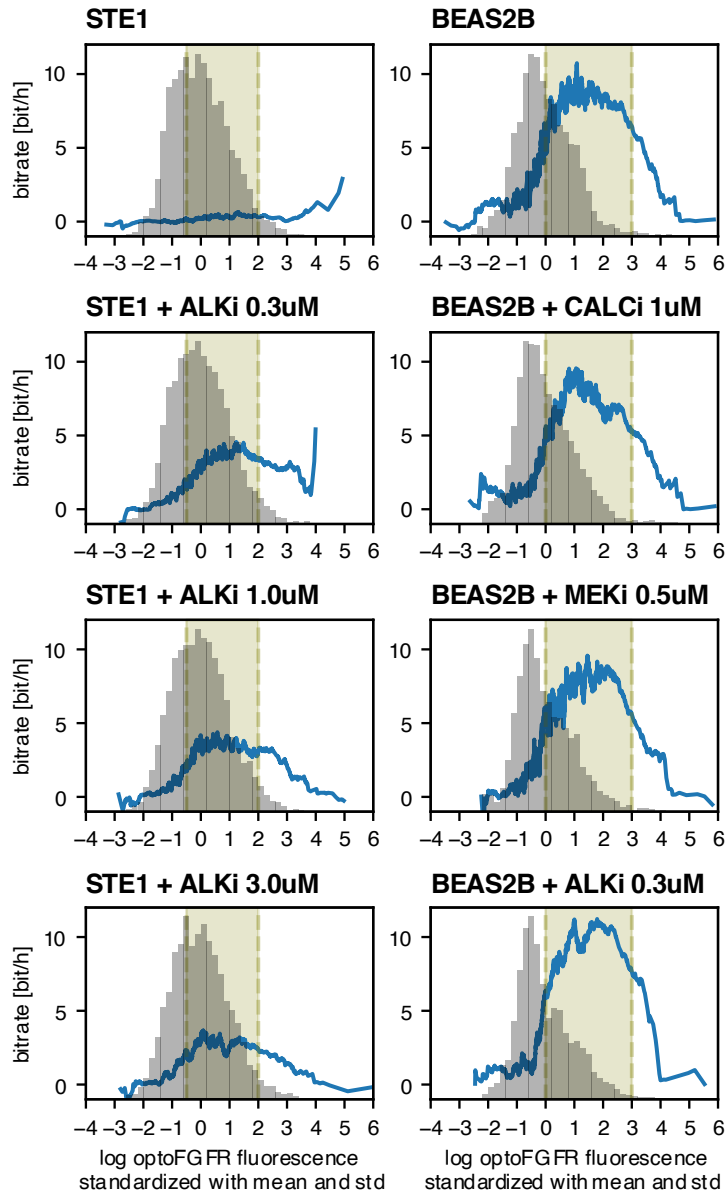
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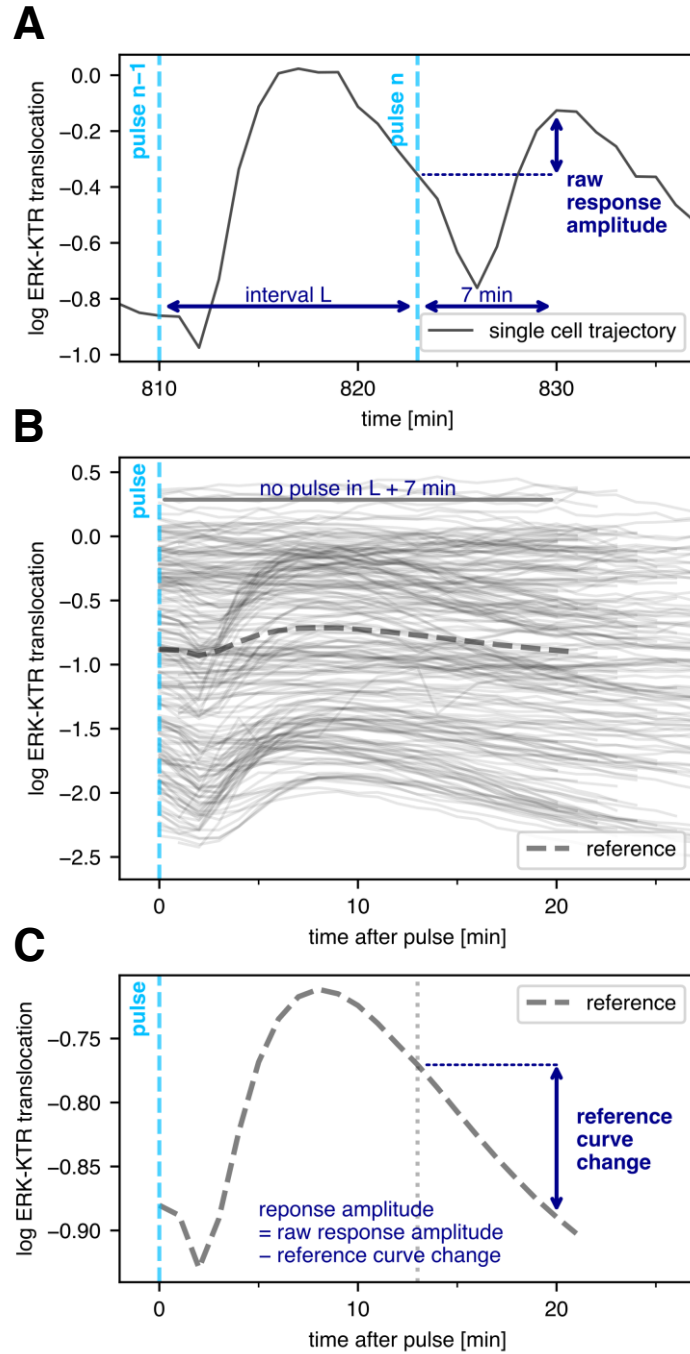
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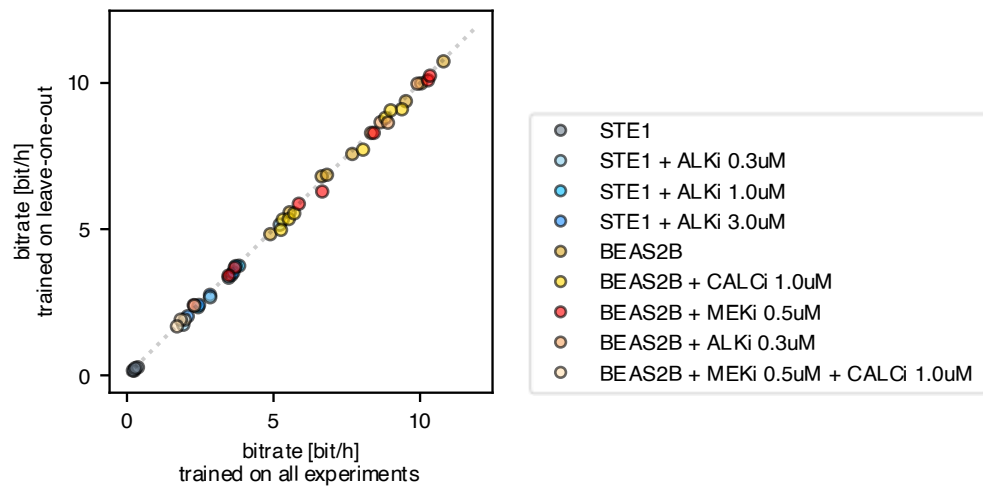
Supplementary Figure S1. Cell preselection based on optoFGFR receptor level.

Average bitrate in individual cells as a function of mean optoFGFR fluorescence (rolling mean over single-cell trajectories; trajectories in each rolling window have a combined duration equal to 100 times the experiment's duration), superimposed over the histograms of mean optoFGFR fluorescence in individual cells. OptoFGFR levels were log-transformed and standardized within each replicate separately, then pooled by cell line and condition. Cells in the highlighted range were used in further analysis.



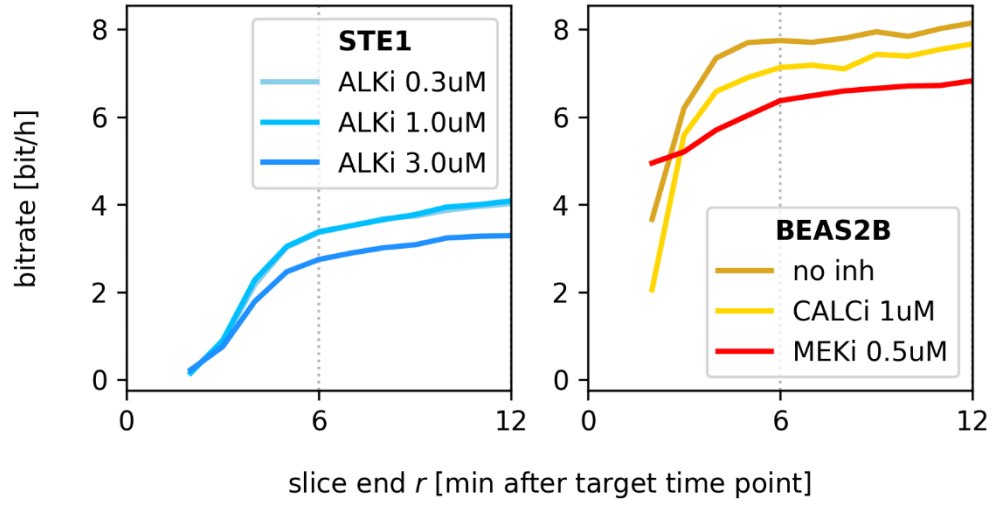
Supplementary Figure S2. Response amplitude definition.

- A To compute the response amplitude to light pulse n , which occurred time L after pulse $(n - 1)$, we first calculated the raw response amplitude as the difference between the ERK-KTR trajectory at pulse and 7 min after.
- B We constructed a reference trajectory by averaging the ERK-KTR trajectories after all pulses not followed by another pulse within the next $L + 7$ min.
- C We computed the change in the reference trajectory between time L and $(L + 7 \text{ min})$ after the pulse. The response amplitude was calculated by subtracting the obtained value from the raw response amplitude.



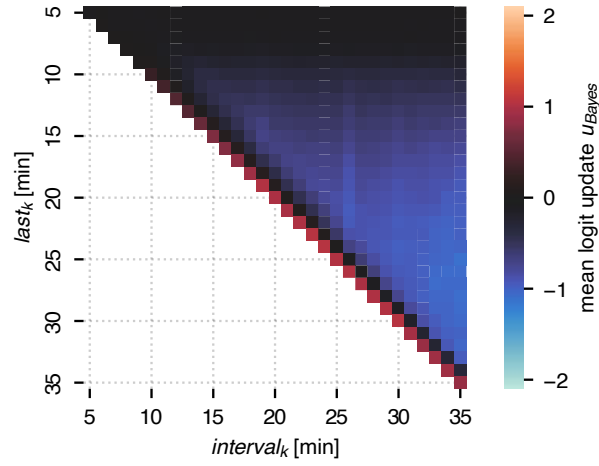
Supplementary Figure S3. Leave-one-out network evaluation.

Average bitrate in particular replicates, computed based on predictions by MLP trained on all conditions and replicates (as used throughout the paper; x-axis), and on all conditions and replicates except for the currently evaluated replicate (y-axis). Identity line dotted.



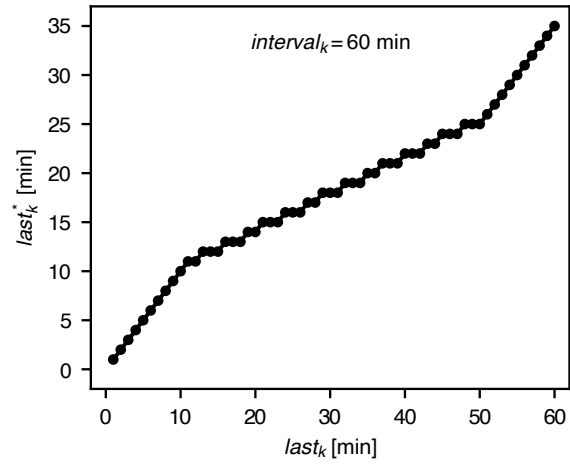
Supplementary Figure S4. Bitrate estimated using MLP trained on slices of increasing length.

The slice used to predict whether there was a pulse at time point k in cell j is $y_{k \dots k+r}^j$, i.e., r consecutive differences $\Delta y_{k+1 \dots k+r}^j$.



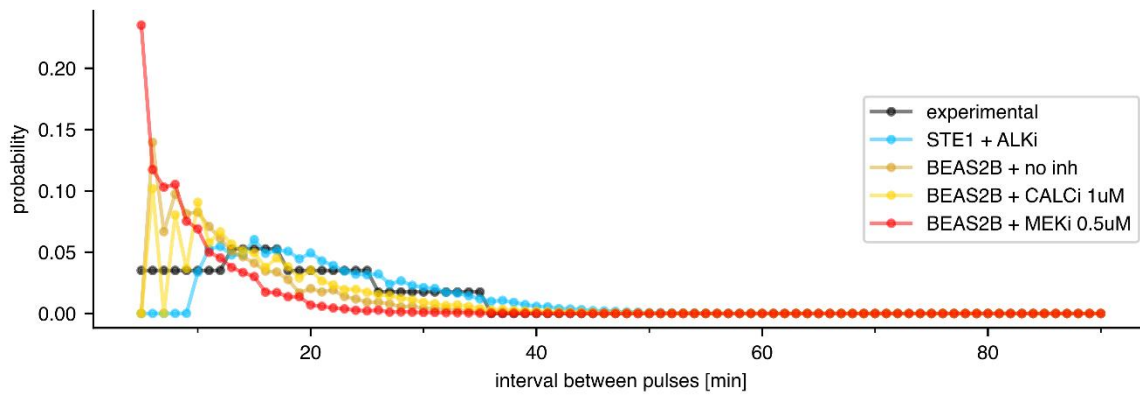
Supplementary Figure S5. Neural network predictions.

Mean logit Bayesian update u_{Bayes} as a function of last_k and interval_k evaluated on STE1 cells with ALKi. The diagonal ($\text{last}_k = \text{interval}_k$) corresponds to timepoints in which a pulse occurred; the u_{Bayes} values on the diagonal are presented in Fig. 6D.



Supplementary Figure S6. Imputation of responses after intervals not present in the dataset.

As intervals longer than 35 min were not present in the experimental sequence, responses for $(interval_k, last_k)$ with $interval_k > 35$ min were sampled from data for $(interval_k^*, last_k^*)$, where $interval_k^* = 35$ min. The plot illustrates the mapping of $last_k$ to $last_k^*$ in the case of $interval_k = 60$ min.



Supplementary Figure S7. Input interval distribution optimized without regularization.

Setup as in Fig. 6E.

Sequence of intervals between light pulses (in minutes):

| 35 | 19 | 30 | 34 | 5 | 14 | 5 | 19 | 33 | 14 | 8 | 6 | 18 ↵
 | 26 | 6 | 15 | 32 | 15 | 15 | 31 | 17 | 29 | 18 | 17 | 14 | 7 ↵
 | 13 | 9 | 10 | 20 | 20 | 9 | 25 | 21 | 7 | 16 | 16 | 27 | 16 ↵
 | 28 | 13 | 21 | 25 | 8 | 17 | 13 | 24 | 10 | 11 | 22 | 22 | 23 ↵
 | 24 | 12 | 23 | 11 | 12 |

Intervals preceding interval of given length:

<i>interval</i>	<i>preceding intervals</i>		
5	14	34	
6	8	26	
7	14	21	
8	14	25	
9	13	20	
10	9	24	
11	10	23	
12	11	24	
13	7	17	28
14	5	17	33
15	6	15	32

<i>interval</i>	<i>preceding intervals</i>		
16	7	16	27
17	8	18	31
18	6	29	
19	5	35	
20	10	20	
21	13	25	
22	11	22	
23	12	22	
24	13	23	
25	9	21	
26	18		

<i>interval</i>	<i>preceding intervals</i>		
27	16		
28	16		
29	17		
30	19		
31	15		
32	15		
33	19		
34	30		
35	∞		

Supplementary Table S1. Sequence of light pulses used in experiments.

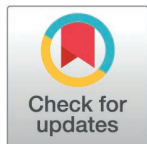
RESEARCH ARTICLE

Information transmission in a cell monolayer: A numerical study

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Abstract

Motivated by the spatiotemporal waves of MAPK/ERK activity, crucial for long-range communication in regenerating tissues, we investigated stochastic homoclinic fronts propagating through channels formed by directly interacting cells. We evaluated the efficiency of long-range communication in these channels by examining the rate of information transmission. Our study identified the stochastic phenomena that reduce this rate: front propagation failure, new front spawning, and variability in the front velocity. We found that a trade-off between the frequencies of propagation failures and new front spawning determines the optimal channel width (which geometrically determines the front length). The optimal frequency of initiating new waves is determined by a trade-off between the input information rate (higher with more frequent initiation) and the fidelity of information transmission (lower with more frequent initiation). Our analysis provides insight into the relative timescales of intra- and intercellular processes necessary for successful wave propagation.

OPEN ACCESS

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Author summary

In biological tissues, traveling waves of cellular activity are observed in the process of wound healing when they coordinate cell replication and collective migration. These waves can carry information over long distances. However, random effects on the single-cell level can affect wave propagation and disrupt information flow. In this paper, using a numerical model we classified these stochastic events and quantified the maximum range and frequency of such waves and their capacity to carry information. We discovered that most effective transmission occurs in relatively narrow channels (formed by directly interacting cells), and that the refractory time, in which a cell is resistant to activation by neighboring cells, must be long with respect to the time needed for cell activation. The optimal time intervals between the initiated waves are of order of few refractory times (depending on channel length).

Introduction

Cells in living organisms communicate through a variety of mechanisms, including chemical and mechanical signals. Long-range communication within a tissue may result from local

Data availability statement: The simulator code is available at <https://github.com/kochanczyk/qeirq>. The code that can be used to reproduce all reported results is available at <https://github.com/pszyk/visavis-seir>.

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Competing interests: The authors have declared that no competing interests exist.

communication between neighboring cells. This is the case for spatiotemporal MAPK/ERK activity waves, originating from the wound edge [1,2] or from leader cells [3] and involving a mechanochemical feedback loop that coordinates collective migration of epithelial cells [3,4]. Excitable ERK activity waves have been shown to control the rate of scale regeneration in zebrafish [5]. These studies highlight the capability of waves to propagate recurrently across successive cell layers despite the inherent discreteness and heterogeneity of the communication medium.

From a dynamical systems perspective, a traveling front may be either an interface between regions in space that are in different equilibria (heteroclinic traveling waves in bistable systems) or an excitation that locally departs and then returns to a unique equilibrium (homoclinic traveling waves in monostable excitable systems) [6]. The heteroclinic traveling waves are formed robustly at the interface between two different regions and as such are resilient to random perturbations [7]. However, the passage of a heteroclinic wave irreversibly changes the state of the reactor that consequently cannot be re-used to support propagation of a subsequent front. In contrast, a homoclinic traveling wave is an out-of-equilibrium “stripe” flanked on both sides with the reactor in the equilibrium state. Although homoclinic traveling waves may be sent recurrently and in this way convey complex messages to spatially distant locations, they are fragile in the presence of stochastic fluctuations. Experimentally, the spatio-temporal ERK signal propagation has been observed to be distorted by random bursts of ERK activity [2,8], which occasionally give rise to spontaneous waves [9].

Here, we sought to quantitatively assess the capacity of a discrete, excitable medium to transmit information encoded within a train of activity waves. Specifically, we investigated stochastic homoclinic fronts propagating in narrow channels formed by directly interacting cells, and determined the efficiency of long-range communication through these channels in terms of the rate of information transmission, also referred to as bitrate. This metric quantifies the amount of information that can be transmitted through a communication channel in a unit of time. We identified several types of stochastic phenomena that reduce the fidelity and thus the rate of information transmission, among which the front propagation failure, new front spawning, and variability in the front velocity were the most impactful. We demonstrated that a tradeoff between the frequencies of front propagation failure and new front spawning determines the optimal channel width, enabling the fronts to reach the greatest distance and maximizing the rate of information transmission. We investigated the system’s ability to relay periodic sequences of fronts as well as transmit binary-encoded information. Binary-encoded information is encoded by specifying a predetermined list of equally spaced time slots and deciding whether a front is initiated or not in each of these time slots. We determined the time interval between the time slots that maximizes the information transmission rate. This optimal time interval or, equivalently, the optimal frequency of time slots results from a tradeoff between the input information rate (higher for on average more frequently initiated fronts) and fidelity of information transmission (lower for on average more frequently initiated fronts). Finally, our exploration of the model parameter space revealed that efficient long-distance information transmission is achievable only if the refractory time is several times longer than the neighbor-to-neighbor activation time.

Results

Model definition

In our continuous-time discrete-space model, each cell in the monolayer assumes one of four distinct states: quiescent (Q), excited (E), inducing (I), or refractory (R), and inter-state transitions follow a predetermined cyclic sequence: $Q \rightarrow E \rightarrow I \rightarrow R \rightarrow Q$ (Fig 1A). When activated by an

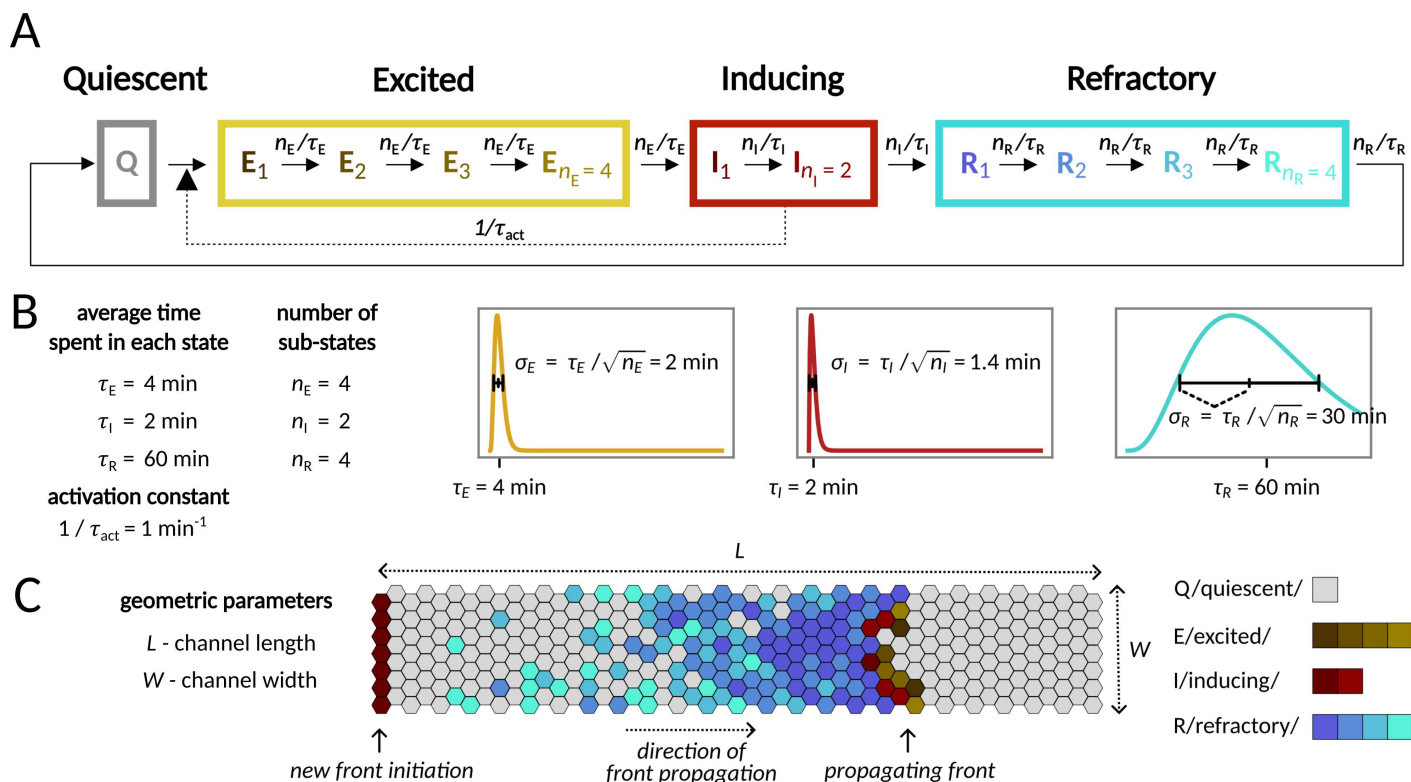


Fig 1. Model. A Scheme of the QEIRQ model with multiple sub-states. A cell in one of the Inducing states (I_1 or I_2) can activate an adjacent cell in the Quiescent state (Q) turning its state to the first Excited state (E_1). Transitions between sub-states are spontaneous first order reactions. **B** Parameters of the model and their nominal values. Total time spent in each of the functional state (E, I, R) has Erlang distribution with scale n_E, n_I, n_R and rate $n_E/\tau_E, n_I/\tau_I, n_R/\tau_R$, respectively, plotted in the graphs. The distributions have means τ_E, τ_I, τ_R and standard deviations $\tau_E/\sqrt{n_E}, \tau_I/\sqrt{n_I}, \tau_R/\sqrt{n_R}$, respectively, as indicated in the figures. **C** Geometry of the reactor used for simulations. Periodic boundary conditions are applied along the longer edge (unless explicitly stated otherwise). A new front is initiated by setting the leftmost layer of cells to the first Inducing state (I_1).

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I neighbor, a Q cell assumes the E state. Subsequent progression from the E to the I state enables the cell to induce activity in its Q neighbors. After the I cell assumes the R state, it loses the ability to activate its Q neighbors and becomes insensitive to activation by neighboring I cells. Finally, reverting to the Q state restores the cell's responsiveness to activation by an I neighbor.

Sequential transition through Q, E, I, and R states and appropriate time scales of the residence in the E, I, and R states are crucial to enable wave propagation. In the context of the MAPK/ERK pathway, the first time scale is associated with signal reception and the signal transduction cascade (through the EGFR/SOS/RAS complex, RAF, and MEK), culminating in ERK phosphorylation; the second time scale is determined by the time required by phospho-ERK to trigger cell contraction that may result in activation of EGFR in neighboring cells [3]; and the third, longest, time scale is related to the refractory period of the signaling cascade, which, due to inhibitory multisite phosphorylation of SOS (by phospho-ERK), is at least partially insensitive to incoming signals.

In the model, the multi-step signal transduction within the MAPK/ERK pathway is reflected by the assumption that the E, I, and R states comprise multiple sub-states (n_E, n_I, n_R , respectively). We assume that each transition between the sub-states (e.g., $E_1 \rightarrow E_2$) is Markovian, and thus the transition times are exponentially distributed (with the rate parameters $n_E/\tau_E, n_I/\tau_I$, or n_R/τ_R ; nominal values of the kinetic parameters are provided in Fig 1B). Consequently,

the total residence times in the E, I, and R states follow Erlang distributions: $\text{ERLANG}(n_E, n_E/\tau_E)$, $\text{ERLANG}(n_I, n_I/\tau_I)$, and $\text{ERLANG}(n_R, n_R/\tau_R)$ with means τ_E , τ_I , and τ_R , respectively. Note that by increasing n_E , n_I , or n_R we reduce the stochasticity of the system, because the variance of an $\text{ERLANG}(n, n/\tau)$ distribution is inversely proportional to n .

In the simulated fully-confluent monolayer, cells are immobile agents arranged on a 2-D triangular lattice of length L and width W (Fig 1C). For the nominal parameter values (Fig 1B), the rates of transitions $Q \rightarrow E_1$ ($1/\tau_{act}$), $I_1 \rightarrow I_2$, and $I_2 \rightarrow R$ (both n_I/τ_I) are all equal. Thus, the probability that an I cell will activate a given neighboring Q cell before transitioning to R is $0.5 + 0.5 \times 0.5 = 0.75$. This value is greater than both the site and the bond percolation thresholds on the triangular lattice, equal 0.5 and ~ 0.347 , respectively [10], permitting front propagation [11].

In the model, we formally assume that all cells are identical and all variability follows from stochastic transitions between cell states. In recent years, it has been demonstrated that population heterogeneity is mainly responsible for observed variability in signal transduction [12–14]. However, in our case (as in many other cases) the two types of noise (intrinsic and extrinsic) have similar consequences. Stochasticity in front propagation is a consequence of variability in state-to-state transition times; this variability may have both intrinsic and extrinsic origins. The key difference is that under intrinsic noise, the front is expected to be perturbed in random locations, while under extrinsic noise, some specific locations in the monolayer will more likely be the source of front perturbation.

Propagation of a single front

Front speed

A propagating front consists of active cells (i.e., cells in either state E or state I) located in its head, followed by a thick block of R cells that prevent backward front propagation. We initiated fronts by setting the states of the cells in the first layer to I_1 and observed the propagation of activity toward the other end of the reactor (Fig 1C).

In the model, the front is deemed to move one step forward once the next cell layer is activated and progresses through all E sub-states to become I. On average, a forward step, in which the front advances by just one cell layer, takes the time $\tau_{act}/\langle n_{neigh,I} \rangle + \tau_E$, where $\langle n_{neigh,I} \rangle$ is the average number of I cells in contact with a single Q cell. In a deterministic model, where the time spent by cells in each state is fixed, the front forms a straight line, and each Q cell at the front head has exactly 2 inducing neighbors. Thus, the inverse propagation speed is $v^{-1}_{deterministic} = \tau_{act}/2 + \tau_E$, which yields 4.5 min/cell layer for the nominal parameter values.

In the stochastic model, however, the front edge becomes rough and the cells to be activated have, on average, more than two I neighbors, which increases the propagation speed. We observed that for the nominal parameter values, the average inverse front velocity $\langle v^{-1} \rangle$ changes with the channel width W from $v^{-1}_{deterministic}$ for $W=1$ to the asymptotic value $v^{-1}_{asymptotic} = 3.5$ min/layer for $W \gtrsim 10$ (Fig A panel a in S1 Appendix).

Transit time and its variance

The expected time in which the front travels the whole channel length L is $\langle \tau_{transit} \rangle = L \langle v^{-1} \rangle$. For sufficiently long channels, the distribution of $\tau_{transit}$ is nearly Gaussian with variance $\sigma^2_{transit} = L \times \sigma^2_0(W)$. The value of σ^2_0 decreases with the channel width, and for $W > 2$ can be well approximated with the formula $\sigma^2_0 = a / W + b$ with $a \approx 6.8$ min², $b \approx 0.4$ min²/layer (Fig A panel b in S1 Appendix). The variance $\sigma^2_{transit}$ critically affects the fidelity of information transmission by determining the precision with which the moment of a front initiation can be inferred from the time at which it reached the end of the channel.

Propagation failure and front spawning

In a stochastic model, a traveling front is subjected to random events disrupting its propagation. In narrow and moderately wide channels, we observed two types of disruptive events: *propagation failure* and *new front spawning* (Fig 2A).

Propagation fails if all E and I cells progress to the R state before exciting any neighboring Q cells (S1 Video). New fronts are spawned when a cell remains in the I state long enough for one of its neighboring cells to recover from R to Q. Such a neighboring cell may get activated and become a source of a new front or fronts (S2 Video). The new front(s) can propagate backward or forward. When a backward-propagating front encounters a forward-propagating front, they collide and usually annihilate (Fig 2B and S3 Video) or, rarely, give rise to another front. For broad reactors (>20 cells wide), fronts may propagate in directions not necessarily parallel to the channel longer axis (Fig B in S1 Appendix and S4 Video), which leads to a chaotic front pattern characteristic for the Greenberg–Hastings model (first defined in Ref. [15] and later recast as a stochastic model and studied, e.g., in Ref. [16]). This 2-dimensional effect is not observed in narrow channels, in which the front tail (the block of cells in the R state at the front's rear side) is longer than the channel width.

We found that the propensity (probability per one cell layer) of a propagation failure event decreases with the channel width W as

$$\lambda_{\text{fail}} = \exp(a_{\text{fail}} \times (W - W_{\text{fail}})), \quad (1)$$

where $a_{\text{fail}} < 0$ and W_{fail} are coefficients that depend on model parameters (Fig 2C). The exponential dependence on W results from the fact that the number of cells in either the E or the I state is proportional to the channel width, and for a front to disappear *all* of them have to simultaneously progress to R without exciting new cells. In contrast, the propensity of new front spawning event increases linearly with channel width as

$$\lambda_{\text{spawn}} = a_{\text{spawn}} \times (W - W_{\text{spawn}}), \quad (2)$$

with a_{spawn} and W_{spawn} dependent on the model parameters. This is because spawning may be triggered by *any* cell across the channel width (Fig 2C).

We noticed that in narrow channels ($W \leq 6$), a single backward front is usually spawned (Fig 2D). In contrast, in broader channels ($6 < W \leq 10$), multiple fronts are often created in a single spawning event. The fronts are typically spawned alternately backward and forward, implying a correlation between the number of fronts generated in either direction. For even broader channels ($W > 10$), long-lasting spawning sites can generate new fronts for prolonged periods, blocking any information transmission. This becomes a dominant disruptive event in very broad channels of $W > 20$ (Fig 2E).

Optimal channel width

In the case of a narrow channel transmitting a series of fronts, the impact of the two types of disruptive events is similar. Propagation failure eliminates the front, whereas a backward-spawned front collides with and annihilates the subsequent (forward-propagating) front. In both scenarios, the total number of fronts received at the other side of the channel is reduced by 1. In narrow channels, a spawning event usually generates a single backward front. Thus, front elimination in a given time span can result either from its propagation failure or from a backward front spawned by the preceding front. We assume that λ_{fail} is small enough so that the probability that the backward front disappears before collision is negligible. Therefore, in narrow channel the total front elimination propensity is roughly

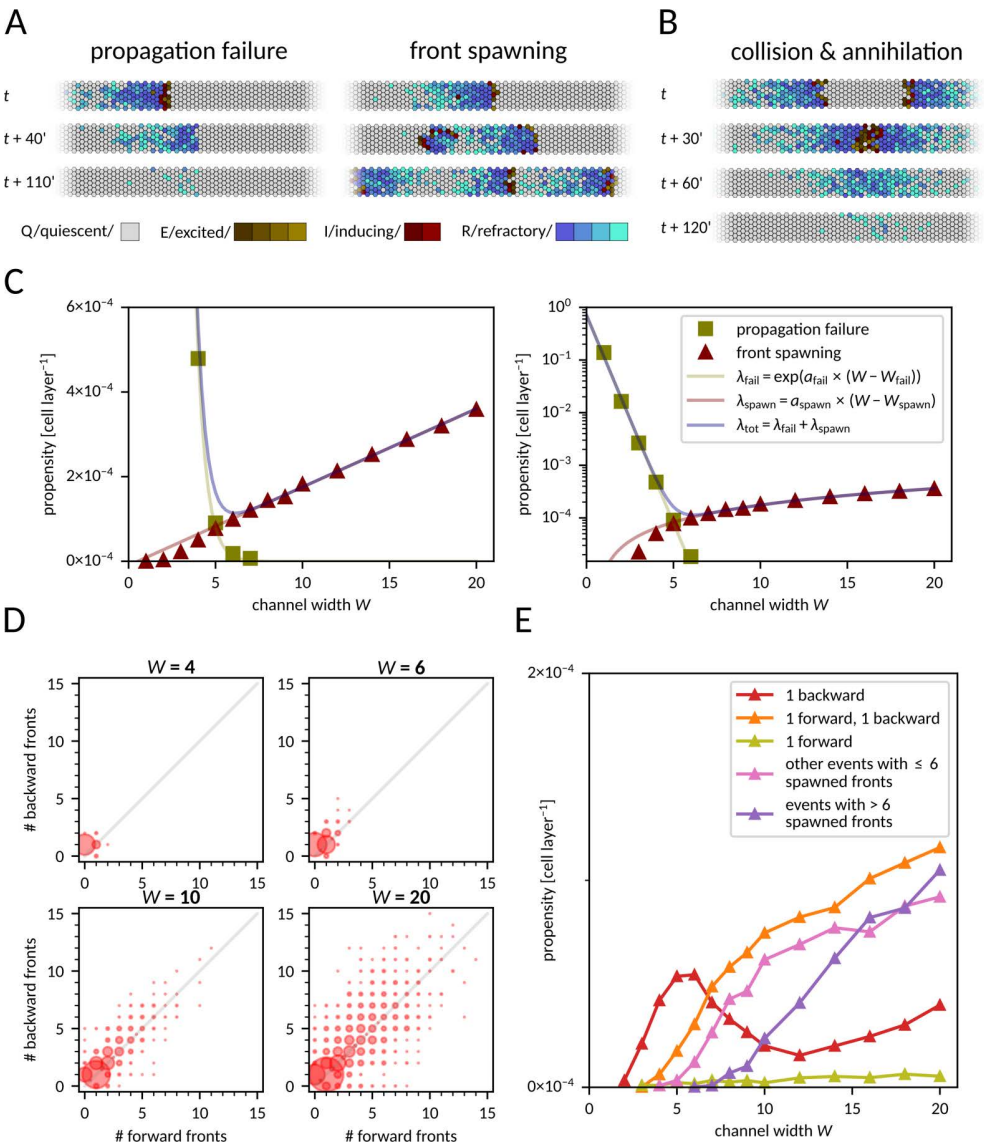


Fig 2. Disruptive events associated with front propagation. A Snapshots from simulations with the two kinds of disruptive events. Left: front dies out as all Excited or Inducing cells turn Refractory before exciting adjacent cells. Right: a single Inducing cell persists long enough for one of its neighboring cells to revert to the Quiescent state. This neighboring cell can then be reactivated, becoming a source of new fronts (in this case, one forward and one backward). See also [S1](#) and [S2 Video](#) for full time-courses. B Two fronts propagating in opposite directions collide and annihilate. See also [S3 Video](#). C Propensity (probability per cell layer) of disruptive events as a function of channel width, in linear (left) and log (right) scale. Linear functions fitted to $\ln(\lambda_{\text{fail}})$ and λ_{spawn} have coefficients $a_{\text{fail}} = -1.82$, $W_{\text{fail}} = -0.19$, $a_{\text{spawn}} = 1.85 \times 10^{-5}$, $W_{\text{spawn}} = 0.53$. D Distribution of the number of forward and backward fronts spawned from a single localized spawning site for four different channel widths. Each disk area is proportional to the probability that a corresponding number of backward and forward fronts was spawned in a single event; the total area of all disks is proportional to the front spawning propensity, which is different for each channel width. E Propensity of spawning one or more fronts in a single localized disruptive event as a function of channel width. Data for panels C–E was gathered from 30,000 simulations for each channel width, channel length was fixed at $L = 300$.

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$$\lambda_{\text{tot}} = \lambda_{\text{fail}} + \lambda_{\text{spawn}}. \quad (3)$$

As λ_{fail} decreases exponentially and λ_{spawn} increases linearly with the channel width, there is an optimal width at which λ_{tot} is minimized. This optimal width maximizes the probability that a given front in a front series passes uninterrupted through the channel. For the nominal parameter values and periodic boundary conditions along the longer channel edges, the optimal channel width is $W_{\text{opt}} = 6$ (Fig 2C), and for this width the average range of uninterrupted front propagation is about 8000 cell layers. As we will see later, the optimum is robust to moderate changes in the values of model parameters. We thus chose the value $W_{\text{opt}} = 6$ as the default channel width for most of the paper. Since uninterrupted front propagation is critical for information transmission, we expected that the information transmission rate would be highest when W is close to W_{opt} .

For non-periodic (inert) boundary conditions, for which interacting cells are bordered by biochemically inert cells, both λ_{fail} and λ_{spawn} are substantially larger because of disruptions initiated at the edges of the reactor. This modifies λ_{spawn} by a constant additive term. Consequently, the minimum value of λ_{tot} is about four-fold higher and attained at $W = 7$ (Fig C in S1 Appendix). As it will be showed later, higher propensities of disruptive events imply lower information transmission rate.

Interaction between fronts

Refractory time

The tail of a front consists of a block of refractory cells, which hinder propagation of the next front. A cell requires, on average, $T_{\text{cycle}} = \tau_{\text{act}} / \langle n_{\text{neigh}_I} \rangle + \tau_E + \tau_I + \tau_R = 66.5$ min to make a full cycle from Q through E, I, and R back to Q (assuming default parameter values and $\langle n_{\text{neigh}_I} \rangle = 2$). In the deterministic model, during this period, the channel is fully blocked; only fronts initiated at a rate smaller than $1/T_{\text{cycle}}$ are transmitted. In the stochastic model, T_{cycle} defines the time scale of the inter-front interval, below which the propensity of disruptive events is significantly elevated, as one can see in the kymographs in Fig 3A.

To investigate how often fronts can be initiated and reliably transmitted, we performed simulations in which pairs of fronts were initiated at various inter-front intervals (Fig 3B). As expected, for intervals shorter than T_{cycle} , propagation of the second front typically fails immediately as the cells at the beginning of the channel have not recovered to Q yet (immediate failure probability is 50% for the interval of 61.5 min). The proximity of the previous front also markedly increases the probability of front spawning. This is likely due to some R cells remaining after the passage of the first front, which recover and become Q only after the passage of the second front. These cells may get activated within the block of R cells in the second front's tail, seeding a new front at the second front's rear side.

The described effects were found to be most significant for inter-front intervals in the range of 50–130 min, i.e., around the value of $T_{\text{cycle}} + \sigma_{\text{cycle}} \approx 96.6$ min, where $\sigma_{\text{cycle}}^2 = (\tau_{\text{act}} / \langle n_{\text{neigh}_I} \rangle)^2 + \tau_E^2/n_E + \tau_I^2/n_I + \tau_R^2/n_R \approx (30.1 \text{ min})^2$ is the variance of the time of the full cycle $Q \rightarrow E \rightarrow I \rightarrow R \rightarrow Q$. The time $T_{\text{cycle}} + \sigma_{\text{cycle}} =: T_R$ can be considered the effective refractory time. For inter-front intervals longer than 130 min, the propensities of disruptive events approach the single-front values (shown in Fig 2C). For intervals shorter than 50 min, the propensities of disruptive events remain high, but the overall probability of their occurrence becomes low, due to the high chance of immediate failure, which we count separately.

Propagation of periodically initiated fronts

The refractory time forces subsequent fronts to not travel too closely, and consequently the average interval between fronts reaching the end of the channel has a minimum (with respect

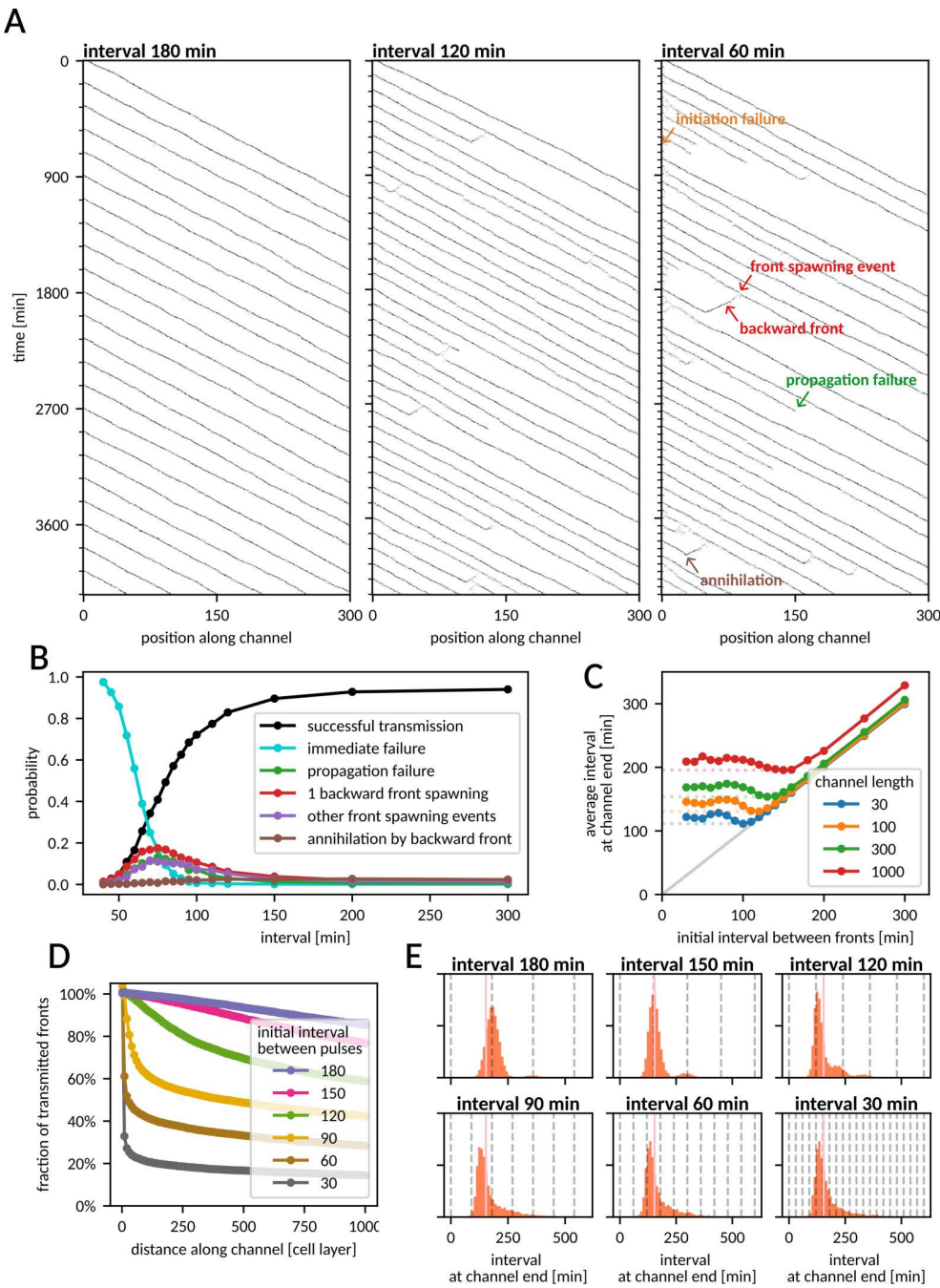


Fig 3. Occurrence of disruptive events as a function of the interval between fronts. **A** Kymographs of the number of active cells (E or I) as a function of time and distance along the channel or fronts initiated periodically at different intervals. Channel dimensions: $W=6$, $L=300$. **B** Probability of disruptive events for the latter of two fronts, as a function of the (initial) inter-front interval. Channel dimensions: $W=6$, $L=300$. **C** Average interval between fronts reaching the end of the channel as a function of the initial inter-front interval for different channel lengths. For each channel length, $T_{trans-min}$ is marked with dotted lines. Channel width $W=6$, length as indicated. **D** Percentage of fronts that reached a certain distance along the channel for different initiation frequencies. Channel dimensions: $W=6$, $L=1000$. **E** Distribution of the interval between fronts that reached the end of the channel for different initial intervals. Dashed vertical lines denote time points at which fronts were initiated. Pink vertical line shows $T_{trans-min}$. Channel dimensions: $W=6$, $L=300$. In all panels, channel width $W=6$. Data for panel B from 3000 simulations for each data point; data for panels C–E from 30 simulations of 500 fronts for each interval.

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to the frequency of front initiation), which we denote $T_{\text{trans-min}}(L)$. In a short channel of length $L = 30$, the effective refractory time T_R is a good approximation of $T_{\text{trans-min}}$ (Fig 3C and Fig D in S1 Appendix). In a long channel, $L = 1000$, we observe that $T_{\text{trans-min}}$ is about 200 min and is achieved when fronts are initiated every 160 min, while a more frequent front initiation results in a slightly longer average front arrival interval. In long channels, a considerable percentage of fronts sent at periods shorter than T_R is eliminated shortly after initiation (Fig 3D). For fronts sent every 150 or 180 min, the propensity of front elimination is initially low, but increases slightly with the distance from the initiation site (Fig 3D). This is because for such intervals disruptive events are relatively rare, but due to fluctuating velocities some fronts draw closer together, and thus the likelihood of disruptive events increases. Consequently, in a channel of length $L = 300$, fronts initiated every 150 or 180 min arrive with time spans distributed around the initial period (Fig 3E, top row), whereas fronts sent with periods $< T_R$ arrive with time spans distributed more broadly around $T_{\text{trans-min}}$ regardless of the initial pulse frequency (Fig 3E, bottom row). Overall, we found that in the limit of short channel lengths, the empirically estimated refractory time T_R sets an upper limit on the transmittable front frequency, while in longer channels, the maximum transmission frequency is lower, and lower initiation frequencies enable propagation of more coherent front trains.

Information transmission rate

Numerical results

To estimate the bitrate, we used a simple binary protocol in which a sequence of equiprobable (probability of a '1' $q = 1/2$) binary symbols $S_i \in \{0, 1\}$ is directly translated into a sequence of fronts ($1 \rightarrow$ front initiated, $0 \rightarrow$ no front initiated) and sent through the channel at regular time slots, with inter-slot interval T_{slot} . We performed simulations and registered the time points at which the fronts reached the end of the reactor. For each time slot t_{slot} , we computed the expected arrival time $t_{\text{expected}} = t_{\text{slot}} + \langle \tau_{\text{transit}} \rangle = t_{\text{slot}} + L \langle v^{-1} \rangle$ and selected the front that arrived closest to t_{expected} . This could be a successfully transmitted front initiated in the considered slot, a front sent in another slot, or a front spawned in a disruptive event. We recorded the difference $\Delta t = t_{\text{arrival}} - t_{\text{expected}}$ between the closest front's arrival time t_{arrival} and t_{expected} . Using all slots in the sequence, we estimated the mutual information per slot (MI_{slot}) as mutual information between Δt and the corresponding binary symbol S (see Methods for details). In Fig E in S1 Appendix we show histograms of Δt for $S = 1$ and $S = 0$. For $T_{\text{slot}} = 150$ min these histograms are well separated, while for $T_{\text{slot}} = 90$ min there is a significant overlap. This implies a higher MI_{slot} for $T_{\text{slot}} = 150$ min. Finally, we calculated the information transmission rate $r = MI_{\text{slot}}/T_{\text{slot}}$. The results for various channel lengths and inter-slot intervals are presented in Fig 4A.

As one can observe, information transmission is highest for moderate values of T_{slot} . For long T_{slot} , the fraction of successfully transmitted information (equal to MI_{slot} measured in bits, as each binary symbol carries one bit of information) is determined by the distant-front dynamics and is thus roughly independent of T_{slot} (Fig 4B). For large values of T_{slot} we may define two regimes:

- (1) The free-front regime, in which the interval between slots is at least twice longer than the transit time. In this regime, fronts spawned backward cannot collide with subsequent fronts, because they reach the beginning of the channel and disappear before the subsequent front is initiated. Consequently, information transmission is determined by the single-front propagation failure propensity. This regime is characteristic of short channels or very long inter-slot intervals.
- (2) The distant-front regime, in which fronts are frequent enough to be annihilated by backward fronts spawned by their predecessors, yet still maintain sufficient distance to avoid

direct interaction (for the default parameters and $L = 1000$ this means $T_{\text{slot}} > 200$ min, see Fig 3C). In this regime, the propensity of disruptive events is still equal to that observed for single fronts, but information transmission is limited by the total disruptive event propensity, as both propagation failure and backward front spawning lead to extinction of one forward front.

For the considered set of parameters, information transmission efficiency (MI_{slot}) is substantially higher in the free-front than in the distant-front regime, because the annihilation of forward-propagating fronts by spawned backward-propagating fronts (Fig 2D) is the main limiting factor in the distant-front regime. In both regimes, MI_{slot} is nearly independent of T_{slot} , and thus the bitrate (equal to $MI_{\text{slot}}/T_{\text{slot}}$) decreases as $1/T_{\text{slot}}$. On the other hand, for short inter-slot intervals the bitrate is limited by strong interactions between fronts (increasing propensity of disruptive events) and transit time dispersion comparable to T_{slot} . Thus, for each channel length, there is an optimum inter-slot interval T_{opt} for which the bitrate is the highest.

For the nominal parameter values and channel length $L = 30$, the optimum is located at $T_{\text{opt}} \approx T_R \approx 96.6$ min, and the maximum bitrate is ~ 0.5 bit/h. Unsurprisingly, the bitrate decreases with an increasing channel length L regardless of T_{slot} due to the accumulation of disruptive effects (Fig 4A and 4C). The optimal interval T_{opt} increases with L , and in the investigated range of channel lengths, the increment is roughly proportional to $\sqrt{L}$ (Fig 4D), which can be attributed to accumulation of transit time variance.

The maximum information rate depends on the channel width (Fig 4E). As expected, the highest bitrate is observed for $W = 6$, for which the total disruptive event propensity is lowest (as shown Fig 2C). In short channels ($L = 30$), bitrate is a slowly decreasing function of W . On the contrary, in longer channels ($L \geq 100$), bitrate decreases faster and drops nearly to zero when long-lasting spawning blocks any regular front propagation. This implies that in the tissue, information can reach relatively short distances from a wound, unless the fronts are confined to narrow structures, such as capillary vessels. The optimal inter-slot interval grows with W (Fig 4F), indicating that in broader channels, in which spawning events are more likely, the reliability of information transmission gained by increasing the distance between fronts (which reduces spawning propensity, Fig 3B) is worth the cost of the reduced front frequency.

Semi-analytical predictions based on phenomenological analysis

There are two major phenomena that set bounds on the information transmission rate: disruptive events and transit time stochasticity. If the intervals between slots are long, the major limiting factor is the possibility of front extinction, due to either propagation failure or a collision with a spawned backward-propagating front. In this case, the amount of transmitted information per slot can be obtained from the confusion matrix with the formula $MI_{\text{slot}} = 1 - 1/2 \times [(p + 1) \log_2(p + 1) - p \log_2 p]$, where p is the probability of front extinction (see Methods for details). To determine the value of p , we used the propensities λ_{fail} and λ_{spawn} of disruptive events for single fronts (Fig 2C) and estimated the expected number of backward fronts spawned in a single event based on results shown in Fig 2D. We were then able to obtain a satisfactory prediction of MI_{slot} in the distant-front limit ($T_{\text{slot}} \rightarrow \infty$) as shown in Fig 4B. By replacing the distant-front event probabilities with estimates for finite interval between fronts from Fig 3B we obtained satisfactory predictions of the bitrate for $T_{\text{slot}} > 120$ min for $L = 100$ and $L = 300$ and for $T_{\text{slot}} > 200$ min for $L = 1000$ (Fig 4G–4I, dotted line).

The prediction taking into account only the disruptive events is satisfactory for long intervals and/or short channels. When T_{slot} is comparable to or shorter than the standard deviation of the transit time, σ_{transit} , fronts reaching the end of the channel may be assigned to a wrong slot. The value of σ_{transit} scales proportionally to $\sqrt{L}$, which makes the misassignment more

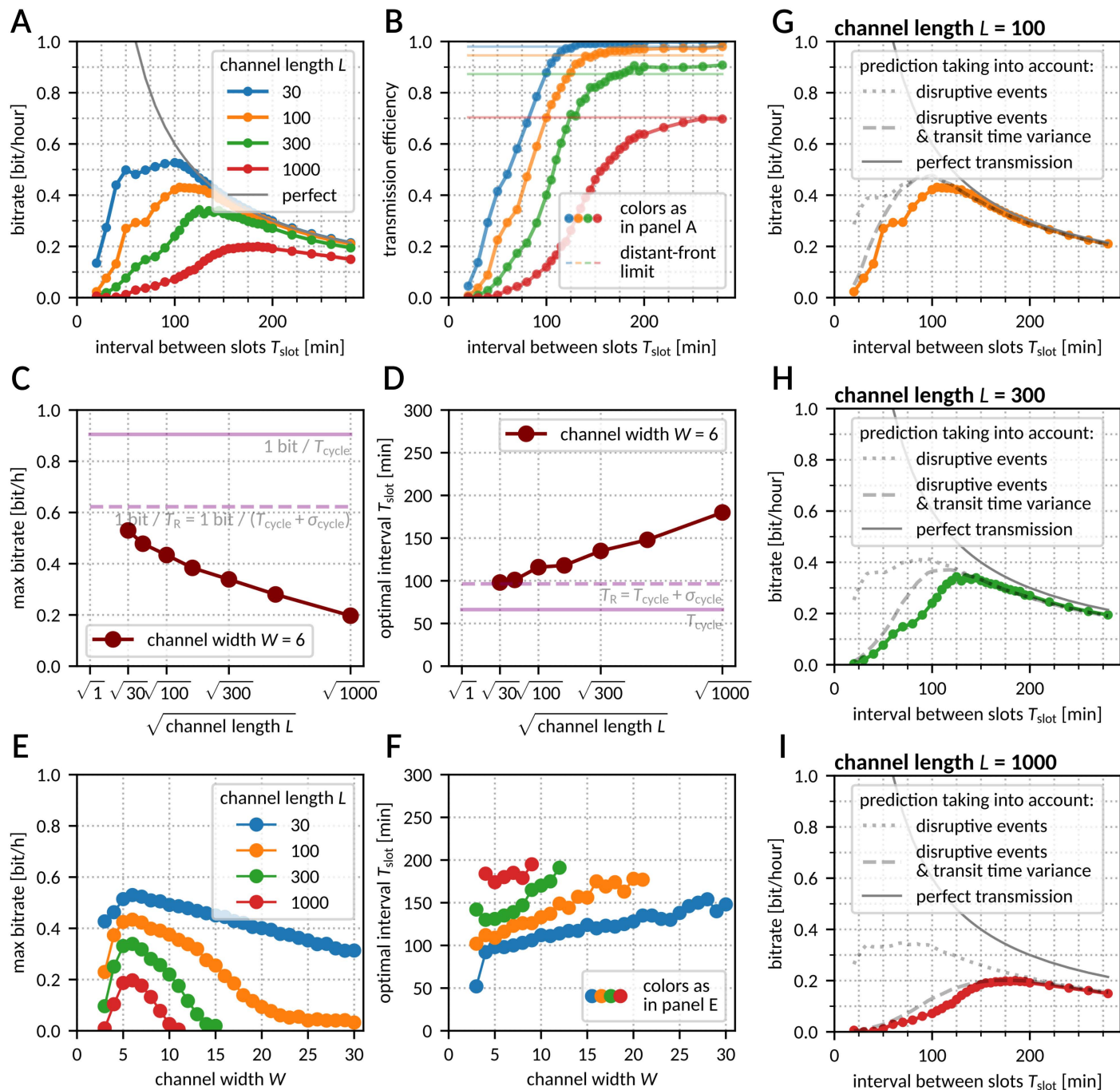


Fig 4. Rate of information transmission. **A** Bitrate as a function of the interval between slots for various channel lengths. The sent information rate is plotted in gray. Channel width $W = 6$. **B** Bitrate from panel A as a fraction of the sent information rate. Solid lines show the information transmission efficiency computed based on distant-front simulations, with a correction for backward fronts hitting the channel beginning (see Methods for details). **C, D** Maximum bitrate (**C**) and optimal inter-slot interval (**D**) as a function of (the square root of) the channel length. Channel width $W = 6$. **E, F** Maximal bitrate (**E**) and optimal inter-slot interval (**F**) as a function of the channel width for various channel lengths. **G–I** Prediction of bitrate taking into account the probability of front extinction due to propagation failure or collision with a backward front (dotted line), and the chance that a front was attributed to a wrong slot due to transit time stochasticity (dashed lines) – see Methods for details. Colored lines as in panel A. Data for panels A–B and G–I from $N = 100$ simulations with 500 front slots for each data point. Data for panels C–F was computed by searching for the maximum in a series of values computed as in panel A.

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likely for longer channels. Once this effect is taken into account (see Methods for details), the prediction (Fig 4G–4I, dashed line) becomes satisfactory both for short and long T_{slot} , with some discrepancy for intermediate T_{slot} values due to fronts spawned forward and other neglected factors.

In all the analyses, for sake of simplicity we employed the binary encoding protocol with equiprobable input symbols ($q = \frac{1}{2}$) and varying T_{slot} . As discussed in Text A in S1 Appendix, in cases where the effective refractory time T_R is large compared to σ_{transit} (e.g., for short channels or long τ_R), the optimal q can be substantially smaller than $\frac{1}{2}$, and consequently the maximum bitrate can be higher. However, only for $L = 30$ the bitrate increase was found significant (+20%).

Thus far, the bitrate was estimated based on the arrival times of individual fronts. We show and discuss the inference based on two consecutive fronts, which yields higher bitrate estimates for short channels ($L = 30$), in Text B in S1 Appendix.

Sensitivity analysis

Disruptive events in single fronts

The maximum front frequency and information transmission rate depend on model parameters. In Fig 5A we analyze how the kinetic model parameters influence the propensities of disruptive events, λ_{fail} and λ_{spawn} , given in Eqs (1–2). For the nominal parameter values, $W_{\text{fail}} \approx 0$ and $W_{\text{spawn}} \approx 1$, and numerical analysis indicates that these coefficients remain in the range $(-1, 2)$ for the considered range of model parameters (Fig F in S1 Appendix). Thus, changes in these coefficients have modest effect on λ_{fail} and λ_{spawn} . Therefore, crucial for understanding the system’s behavior are the changes of coefficients a_{fail} and a_{spawn} . It is important to notice that a_{fail} changes approximately linearly with the kinetic parameters, while a_{spawn} scales exponentially; as a consequence, both λ_{fail} and λ_{spawn} exhibit exponential dependence on τ_E , τ_I , τ_R , and τ_{act} .

We may notice that a_{fail} (and thus λ_{fail}) does not depend on τ_E and τ_R , as those parameters have no influence on whether a cell will be activated. The failure propensity increases with τ_{act} and decreases with τ_I , because increase of the ratio τ_{act}/τ_I implies a lower probability that an I cell activates a Q neighbor before proceeding to R.

The coefficient a_{spawn} (and thus λ_{spawn}) depends on all four kinetic parameters. It increases with τ_{act} , which can be explained as follows: high τ_{act} implies low cell activation propensity, which renders some cells activated at the rear side of the front and mediating front spawning. Importantly, the value of coefficient a_{spawn} increases with τ_E (the increase of which implies a higher chance that the cell becomes inducible in the front tail) and decreases with τ_R (the increase of which implies a broader zone of R cells behind the front). Coefficient a_{spawn} decreases with n_E , n_I , and n_R , because the increase of the number of intermediate states renders the distribution of times of transitions $E \rightarrow I$, $I \rightarrow R$, and $R \rightarrow Q$ narrower, resulting in less stochastic front propagation. Consequently, the minimum value of λ_{tot} is an increasing function of τ_E , decreasing function of τ_R , and decreasing function of n_E , n_I , and n_R (Fig 5B). Another consequence of the discussed dependence of a_{spawn} on τ_E and τ_R , and independence of a_{fail} of these two kinetic coefficients, is that an increase of τ_R and/or decrease of τ_E shift W_{opt} to higher values (Fig 5C).

In summary, we showed that an increase of the τ_R/τ_E ratio (i.e., the ratio of the residence time in the refractory state to the residence time in the excited state) nearly exponentially reduces the disruptive event propensity λ_{tot} , and linearly increases the optimal width W_{opt} of the channel.

Periodic fronts and bitrate

Understanding how the model kinetic parameters influence the propensities of disruptive events allows us to analyze and interpret their impact on $T_{\text{trans-min}}$ and maximum bitrate (Fig 6). The

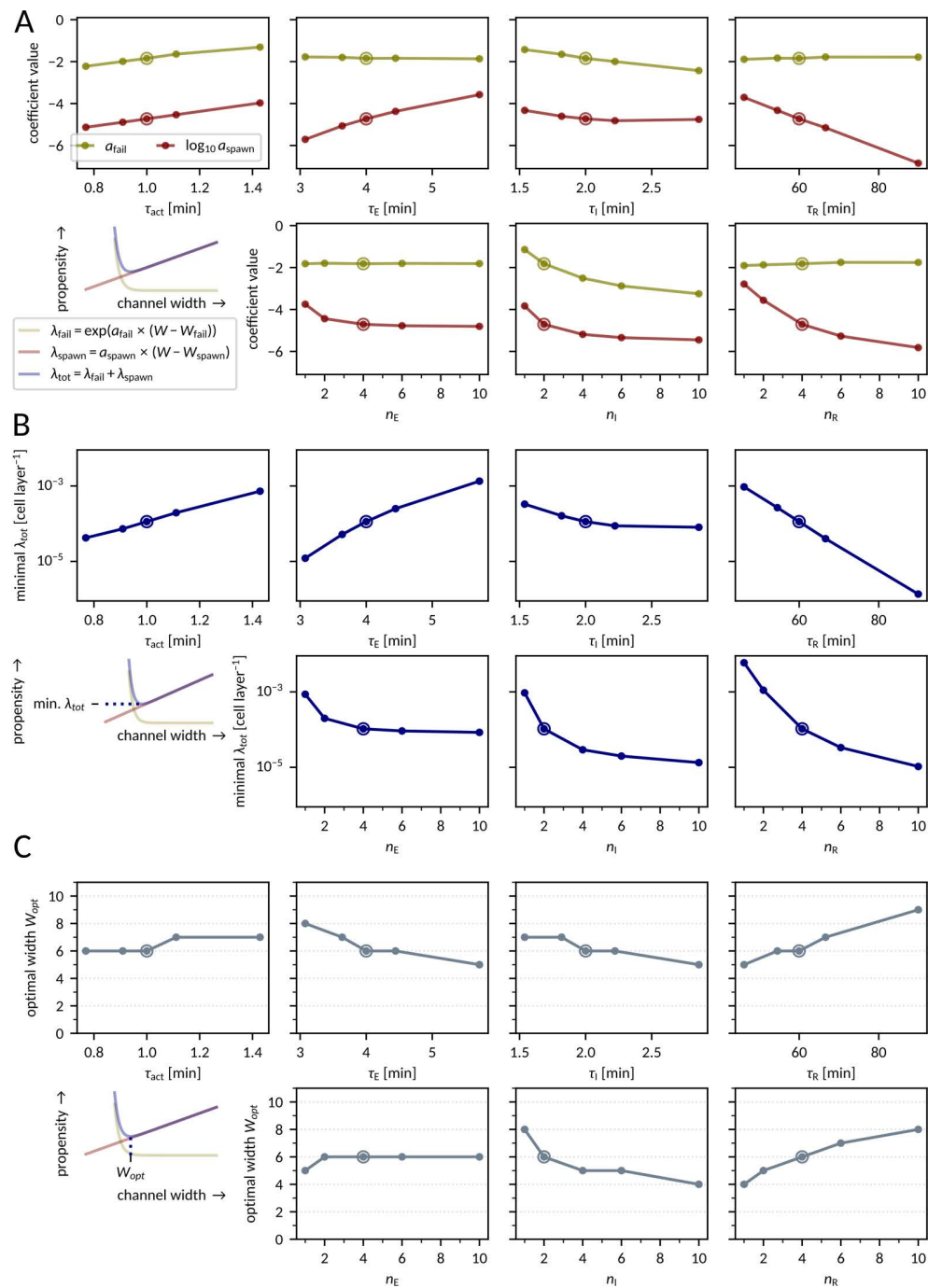


Fig 5. Sensitivity analysis – disruptive events in single fronts. **A** Dependence of slope coefficients a_{spawn} and a_{fail} on model parameters. **B** Dependence of the minimum total disruptive event propensity λ_{tot} on model parameters. **C** Dependence of the optimal channel width W_{opt} on model parameters. Each data point was obtained by fitting $\lambda_{\text{spawn}}(W)$ and $\lambda_{\text{fail}}(W)$ curves to numerical data as in Fig 2C. The encircled dots in each panel correspond to the nominal parameter set given in Fig 1B. All parameters apart from those indicated on the horizontal axis are fixed to their nominal values.

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dependence of $T_{\text{trans-min}}$ on the on the τ_{act} and τ_i is dictated by the dependence of λ_{fail} and λ_{spawn} on these parameters; as expected, the increase of λ_{fail} or λ_{spawn} leads to the increase of $T_{\text{trans-min}}$. The dependence of $T_{\text{trans-min}}$ on τ_E and τ_R is more intriguing: $T_{\text{trans-min}}$ has maxima with respect to τ_E and decreases for large τ_E ; $T_{\text{trans-min}}$ decreases also for small τ_R . This is puzzling, as we know from Fig 5A that large large τ_E and small τ_R imply large λ_{spawn} , which could block front propagation. However, as we can see in kymographs in Fig 6B and 6C, for small τ_R and large τ_E we observe a distinct pattern of front spawning. In this mode, numerous backward and forward fronts are created, so the front density (especially for small τ_R) at the end of the channel is higher than at its beginning. In this regime it is impossible to coordinate collective cell motion in the direction of the front source.

Kymographs in Fig 6B also suggest that there should exist some optimal τ_R , at which the bitrate is highest. In fact, this maximum is attained close to the nominal value of τ_R , which is 60 min (Fig 6D). Larger values of τ_R do not allow for frequent fronts, while smaller ones are associated with a spawning front propagation pattern (blocking any information transmission). For the same reason, information transmission is blocked for large values of τ_E . The information transmission rate increases monotonically with decreasing τ_E . This is because the decrease of τ_E reduces λ_{spawn} and to some extent the refractory time, and does not influence λ_{fail} .

Unsurprisingly, in the MAPK/ERK pathway, τ_E is short (several minutes) despite ERK activation being a multistep process. As shown in Fig G in S1 Appendix, the maximum bitrate grows monotonically with the number of sub-states (providing that the total time of all sub-states remains constant). In agreement with the influence of τ_{act} on λ_{fail} and λ_{spawn} , the bitrate decreases monotonically with τ_{act} , and, similarly as with τ_E , τ_{act} appears to be short for the MAPK/ERK pathway. Finally, the bitrate attains its maximum close to the nominal value of τ_i .

Front propagation in broad channels

In broad channels of $W \gtrsim 15$, front spawning events frequently seed multiple fronts (Fig 2D and 2E) giving rise to chaotic front propagation patterns (as shown in Fig B in S1 Appendix and S4 Video), which can block information transmission for very long times. Thus, for such channels information transmission rate is close to zero, and one should rather ask about the number of fronts that can be transmitted before chaotic patterns develop due to spawning event(s). For this reason, we determined the expected number of fronts N_{fronts} that can propagate through the channel of $L = 300$ and $W = 6, 15, 30$, and 60 before the first spawning event. In Fig 7, lines correspond to noninteracting fronts ($\lambda_{\text{spawn}}^{-1}$ computed based on the a_{spawn} and W_{spawn} estimation shown in Fig 5A and Fig F in S1 Appendix), while circles were obtained from simulations with two fronts separated by the interval of $4 \tau_R$. One can see that (for $L = 300$) such interval is sufficient to neglect the influence of inter-front interaction on spawning.

$N = 3000$ simulations were performed for each data point. Encircled points correspond to the nominal parameters given in Fig 1B.

We may notice that N_{fronts} is a nearly exponentially increasing function of τ_R (Fig 7A) and a nearly exponentially decreasing function of τ_R (Fig 7B). Therefore, although N_{fronts} decreases with the channel width as $1/W$ (for the nominal parameters, N_{fronts} is about 10 times higher for $W = 6$ than for $W = 60$), the effect can be compensated with a relatively small change in the kinetic parameters. For example, for $W = 60$, increasing the refractory time to $\tau_R = 90$ min results in the increase of N_{fronts} about 50 times, to over 100. Because for $W > 10$ the propensity of front propagation failure is negligible (Fig 2C), all initiated fronts will reach the end of the channel. Additionally, the transit time variance $\sigma^2_{\text{transit}}$ is a decreasing function of W (Fig A panel b in S1 Appendix), so for $W > 10$, $L = 300$ the distribution of the transit time is also

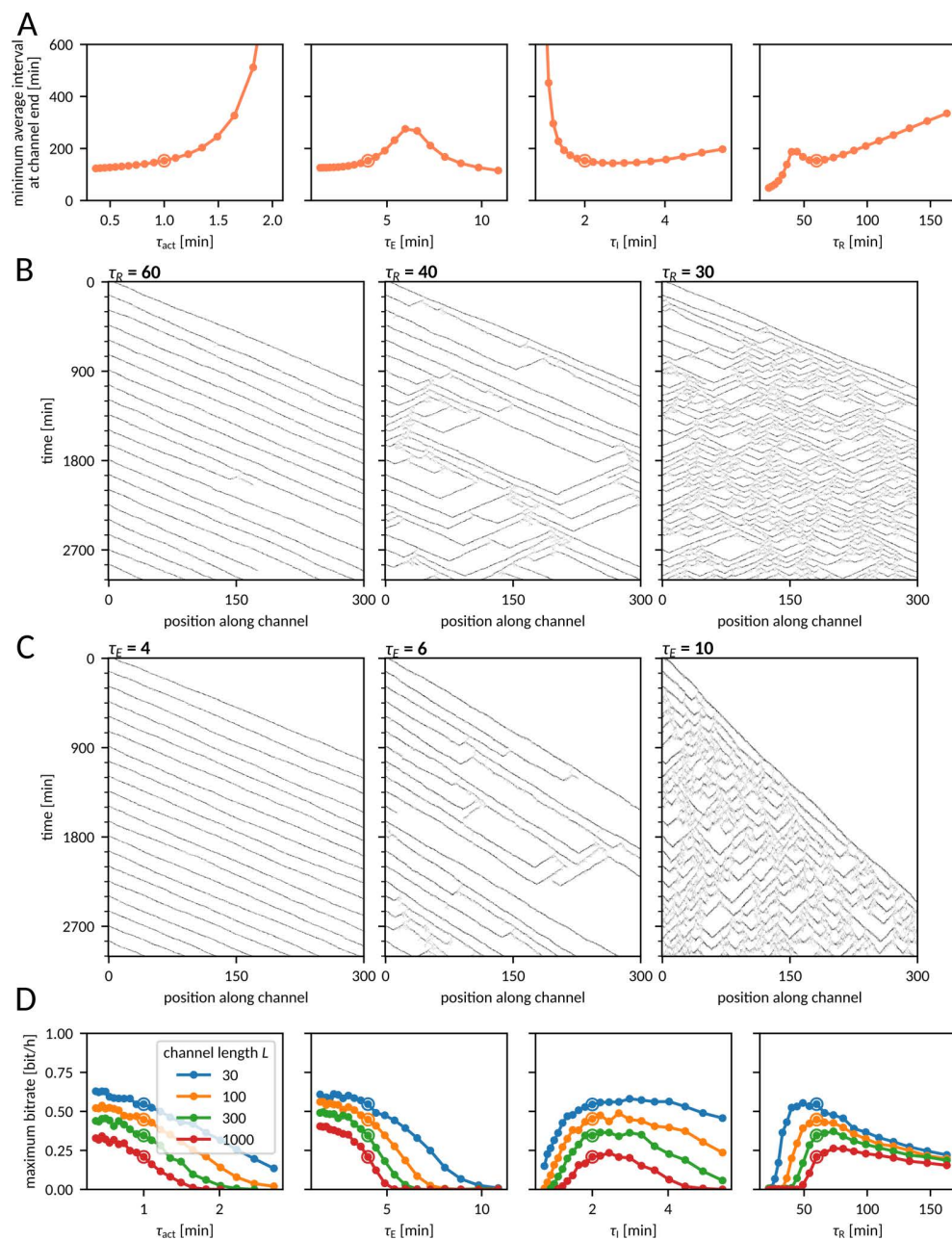


Fig 6. Sensitivity analysis – periodic fronts and the bitrate. A Interval $T_{\text{trans-min}}$ (minimized with respect to the initial interval) at the channel end of the channel as a function of the model parameters. The remaining parameters are equal to the nominal values given in Fig 1B. B, C Kymographs of the number of active cells (E or I) as a function of time and distance along the channel for fronts initiated periodically at the interval of 150 min for three different values of τ_R (B) and τ_E (C). The remaining parameters are equal to the nominal values given in Fig 1B. D Maximal bitrate (maximized with respect to the input bitrate) as a function of the model parameters. In all panels, channel dimensions are $W=6$ and $L=300$. Encircled dots correspond to the nominal parameters given in Fig 1B.

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sufficiently narrow for the received fronts to be uniquely assigned to their slots (for $T_{\text{slot}} \geq 4\tau_R$). Therefore, for the aforementioned example parameters ($L=300$, $W=60$, $\tau_R=90$ min, and other parameters having nominal values), before the channel is blocked, on average

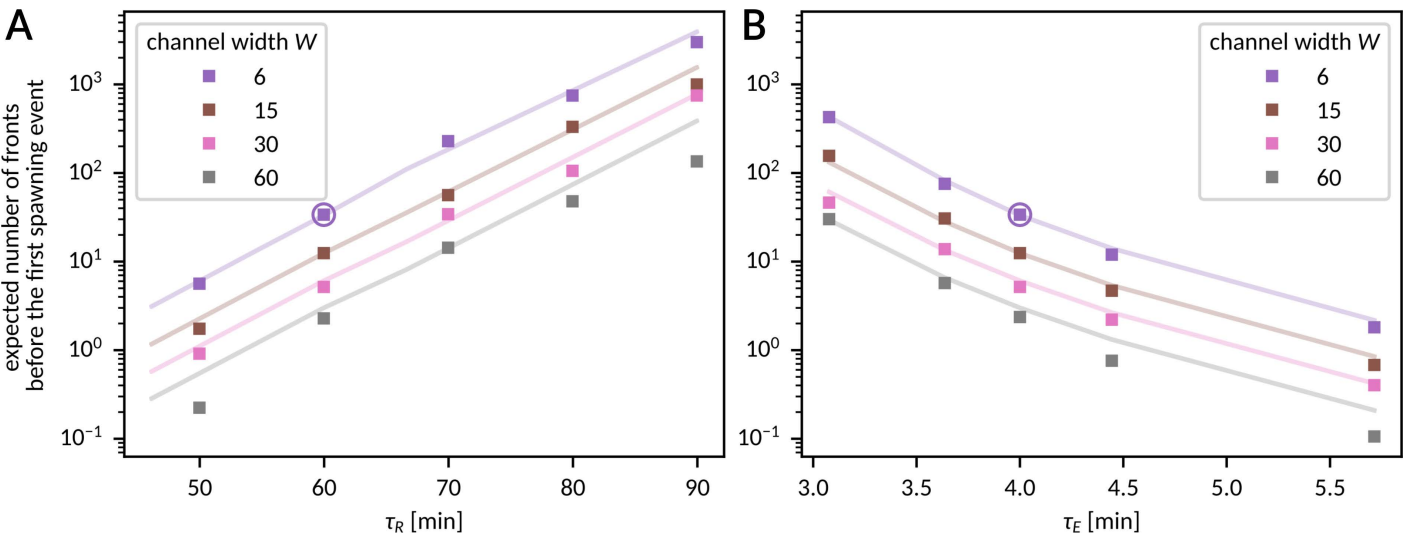


Fig 7. Sensitivity analysis – front propagation in broad channels. A, B Expected number of fronts that will pass uninterruptedly through the channel of length $L = 300$ before the first spawning event as a function of the model parameters τ_R (A) and τ_E (B). Computed based on the spawning probability for the latter of two fronts initiated $4 \times \tau_R$ apart (squares) or based on the coefficients a_{spawn} , W_{spawn} from Fig 5A and Fig F in S1 Appendix (lines).

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$N_{\text{fronts}} \approx 100$ can be sent, received, and properly assigned to their slots, which allows for transmitting 200 bits of information in the binary protocol with equiprobable symbols.

Discussion

ERK activation triggers cell contraction, which leads to activation of EGFR in neighboring cells [3]. In cell collectives, this mechanochemical coupling coordinates the propagation of waves of ERK activity and cell movement against the waves’ direction [9]. As the MAPK/ERK cascade is inhibited behind the front, cell contraction induces subsequent ERK activation in cells directly ahead of the front, rather than behind it. The mechanochemical coupling was theoretically studied in 1-D and 2-D models by Boocock et al. [4,17]. Their model, constrained with data obtained from experiments on MDCK cells but omitting details of signal transduction through the MAPK/ERK cascade, allowed them to determine the optimal wavelength and period for maximizing migration speed towards the tissue boundary. In our study, we investigated the processes that interfere with the stochastic propagation of activity waves.

Canonically, trigger (homoclinic) traveling waves employ a positive feedback to propagate over long distances [18–20]. In our model, the positive feedback (at the tissue level) arises when the inducing cell excites a quiescent cell, and then the quiescent cell becomes inducing itself. Because after the passage of a homoclinic wave, the system returns to its single steady state, such waves can be initiated recurrently at desired time points; the same property entails that in the presence of stochastic fluctuations, waves can vanish but also may arise spontaneously.

In our study, we characterized two types of disruptive events: front propagation failure and new front spawning. Propagation failure eliminates the front, whereas a single backward-spawned front collides and annihilates with the subsequent front in the series. Thus, when fronts are initiated repeatedly, in both cases, the total number of fronts is reduced by 1. Consequently, the probability that a front in a series of fronts passes through the channel decreases with the total disruptive event propensity $\lambda_{\text{tot}} = \lambda_{\text{fail}} + \lambda_{\text{spawn}}$. Importantly, because λ_{fail} decreases (exponentially), while λ_{spawn} increases (linearly) with the channel width W , there is some

optimal channel width W_{opt} , for which the probability of uninterrupted front propagation through the channel is the highest. This result is surprising because intuitively, the reliability of stochastic signal transduction should monotonically grow with the channel width. The fidelity of front propagation in relatively narrow channels may allow for transmission of ERK waves in narrow structures like capillary and lymphatic vessels, in which sequential ERK activation [21] and calcium waves [22] have been observed.

The tail of each front consists of a layer of refractory cells, which block the propagation of other fronts close behind. The effective refractory time $T_R = T_{\text{cycle}} + \sigma_{\text{cycle}}$, where T_{cycle} is the cell cycle time (from Q through E, I, and R back to Q) and σ_{cycle} is the cell cycle standard deviation. When studying periodically initiated fronts, we observed that the time T_R approximates the lower limit on the time interval between fronts that can be transmitted through a short channel. In longer channels, the minimum average interval between fronts $T_{\text{trans-min}}$ is larger than T_R , and increases with the channel length.

To numerically estimate the rate at which information can be transmitted, we employed a binary encoding protocol. We found that for the optimal channel width, the bitrate is 0.5 bit/hour for $L = 30$ and 0.2 bit/hour for $L = 1000$ for the nominal model parameter values, consistent with the timescales of processes implicated in the MAPK/ERK signaling cascade. There are two major phenomena that limit the information transmission rate: disruptive events and transit time stochasticity. For long intervals between fronts, the possibility of front extinction due to disruptive events is the limiting factor. In this case, we showed that the amount of information transmitted per slot equals $MI_{\text{slot}} = 1 - 1/2 \times [(p+1) \log_2(p+1) - p \log_2 p]$, where p is the probability of front extinction, which is an increasing function of front slot frequency. Since the bitrate is the product of the slot frequency and MI_{slot} , it attains its maximum for some optimal inter-slot interval. When the interval between front slots is comparable to or shorter than the standard deviation of the transit time (that increases with channel length), fronts reaching the end of the channel may be attributed to a wrong slot. Once this effect is taken into account, our phenomenological predictions become satisfactory for both short and long inter-slot intervals. As expected, the bitrate reaches its maximum for the channel width which minimizes the propensity of disruptive events.

We performed a sensitivity analysis to show that the ratio of the refractory to the excited state residence times (τ_R/τ_E) nearly exponentially reduces the total disruptive event propensity. Surprisingly, we found that $T_{\text{trans-min}}$ decreases for large τ_E and small τ_R , attaining high values when the disruptive event propensity is high. Kymographs indicate that for small τ_R or large τ_E there is a distinct chaotic front spawning regime. In this regime, multiple backward and forward fronts are created, so that the front density (especially for small τ_R) at the end of the channel is higher than at its beginning. The existence of multiple backward fronts excludes coordination of collective cell motion in the direction of the signaling source. We found that (for other parameters fixed) there exists an optimal τ_R (close to the nominal τ_R value of 60 min) associated with the highest bitrate. Larger τ_R precludes frequent fronts, while smaller τ_R leads to the chaotic front spawning regime blocking any information transmission. Information transmission is also blocked for large values of τ_E , and bitrate increases monotonically with decreasing τ_E . This is because a decrease of τ_E reduces λ_{spawn} and does not influence λ_{fail} . Unsurprisingly, in the MAPK/ERK pathway, τ_E is short despite ERK activation being a multi-step process.

Finally, we observed that in broader channels, front spawning causes that front propagation becomes chaotic, as observed in the experimental work of Hino et al. [3]. However, sensitivity analysis indicates that front spawning propensity grows only linearly with the channel width while it decreases exponentially with the refractory time. Therefore, a relatively modest increase of the refractory time allows for unperturbed transmission of multiple fronts

also in broader channels before chaotic front patterns develop. This may explain why the ERK activity waves observed in zebrafish scales [5] are much more stable.

Shannon mutual information and information rate give an upper bound on the complexity of decisions that the receiving agent can make in response to the sent signal. It was recently demonstrated that the navigation of *Escherichia coli* in changing chemoattractant gradients is information-limited [23]. For a cell to informally choose among two possible responses, it has to receive at least $\log_2 2 = 1$ bit of information. If a cell is to make such binary decisions every ten minutes, the required information rate is 6 bit/h. Previous studies have established that the NF- κ B and MAPK/ERK pathways are able to transmit merely 1 bit of information about the strength of a stimulus enabling binary decision-making [24,25]. In a recent work, we have established that the MAPK/ERK cascade is capable of transmitting information between membrane (opto)receptor (opto-FGFR) and ERK at bitrate exceeding 6 bit/hour [26]. This high bitrate allows for coordination of fast processes, such as mitotic divisions in *Drosophila melanogaster*, that occur synchronously about every 10 min [27].

To coordinate behavior of a cell population, as in the case of wound healing, information must be propagated between cells, and the bitrate limits temporal resolution, complexity of a desired behavior, and the extent of cell coordination. Travelling fronts (in contrast to diffusion) can propagate signals at a constant speed over long distances. Our analysis of ERK activity waves in cell monolayers indicates that there is an optimal channel width and optimal slot frequency, at which the front propagation is least perturbed and consequently the highest bitrate is achieved. Additionally, sensitivity analysis suggests that the time scales associated with signal transmission within the MAPK/ERK pathway are in the range that allows for efficient information transmission by traveling ERK activity waves.

Methods

Numerical simulations

Kinetic Monte Carlo simulations on a triangular lattice were carried out according to the Gillespie algorithm using the code adapted from Ref. [28]. In all simulations except those for Fig C in S1 Appendix, periodic boundary conditions were applied along the lower and the upper edges. Cells traverse the QEIRQ sequence of (sub)states when activated by a neighboring cell in an I substate. Cells cannot change their locations nor die. State-to-state transitions are shown in Fig 1A and default parameter values are given in Fig 1B.

Front tracking

Front arrival time. Activity (i.e., total number of cells in states E or I, N_{EI}) in the last cell layer was counted as a function of time; local maxima along the temporal axis were found and filtered based on peak height to remove peaks closer than 20 or 30 min apart.

Disruptive event detection. Activity (i.e., the total number of cells in states E or I, N_{EI}) was counted as a function of the distance x from the beginning of the channel and time to form a kymograph ($x-t$ plot; Figs 3A, 6B, and 6C). To determine front positions, the kymograph was smoothed (with a Gaussian kernel along the x -axis and an exponential kernel along the t -axis); then local maxima of N_{EI} along the x -axis were found (thresholds on minimal peak height and minimal distance between peaks were used to discard short-lasting fluctuations). Fronts were tracked using LAPTRACK [29]. Fates of fronts that did not reach the end of the channel were classified as ‘annihilation’, ‘immediate failure’, or ‘propagation failure’ depending on whether, respectively, the front disappeared in the vicinity of another front propagating in the opposite direction, within a certain minimal distance from the channel beginning (1–5 cell layers depending on the channel width), or while propagating freely through the channel. To detect

front spawning, track splits were recorded. Tracks shorter than 50 min that did not reach the channel end nor split into other tracks were discarded together with the split in which they were created. The remaining track splits were treated as front spawning and grouped into events: two splits were assigned to a single spawning event if they occurred not further than 20 cell layers apart in space and 150 min away in time (Fig 2D and 2E).

Numerical data analysis

Front speed and transit time variance. To determine the average inverse front speed and transit time variance (Fig A in S1 Appendix), for each channel width, $N=30,000$ simulations with a single front were performed in a reactor of length $L=300$ (or $L=30$ in the cases when, due to a very short average front range, too few fronts reached the distance of 300 to collect sufficient statistics). Average inverse front speed and transit time variance were computed as $\langle v^{-1} \rangle = \langle \tau_{\text{transit}} \rangle / L$ and $\sigma_0^2 = (\text{Var } \tau_{\text{transit}}) / L$, where τ_{transit} is the time of the first detected front arrival in each simulation. Fronts that did not reach the end of the channel were discarded; therefore, formally, we compute both quantities conditioned on the fact that the front reached the channel end.

Disruptive events – single fronts. For each channel width, $N=30,000$ simulations with a single front were performed in a reactor of length $L=300$; fronts were tracked, and front fates were determined. For each simulation, the first disruptive event (propagation failure or front spawning) was determined and its position was recorded. The number of simulations with at least one disruptive event, n_{event} , and the average position of the first event, x_{event} , were obtained. The total event propensity was calculated with the formula

$$\lambda_{\text{tot}} = \frac{n_{\text{event}}}{n_{\text{event}} \times x_{\text{event}} + (N - n_{\text{event}}) \times L}. \quad (4)$$

Propensities of events of a particular type were computed as

$$\lambda_{\text{fail}} = \frac{n_{\text{fail}}}{n_{\text{event}}} \lambda_{\text{tot}}, \quad (5)$$

$$\lambda_{\text{spawn}} = \frac{n_{\text{spawn}}}{n_{\text{event}}} \lambda_{\text{tot}}, \quad (6)$$

where n_{fail} and n_{spawn} denote the number of simulations in which, respectively, propagation failure and front spawning was the first disruptive event. We used the least squares method for fitting coefficients a_{spawn} , a_{fail} , W_{spawn} , W_{fail} in Eqs (1–2) (Figs 2C, 5A, and Fig F in S1 Appendix). The optimal width W_{opt} in Fig 5C was taken as the integer with minimal λ_{tot} , computed according to Eqs (1–3) based on the best-fit coefficients. Minimal disruptive event propensity (Figs 5B and 6A) was computed using Eqs (1–3) for $W = W_{\text{opt}}$. The estimation of the number of fronts spawned in either direction (Fig 2D and 2E) was based on the first spawning event in each simulation.

Disruptive events – interacting fronts. For each considered inter-front interval, $N=3000$ simulations were performed with two fronts separated by the given (initial) interval; fronts were tracked and their fates were determined. Based on this, the probability of disruptive events concerning the latter front (failure, spawning, or annihilation by a front spawned by the first front) was calculated (Fig 3B).

Front arrival frequency. The average frequency of fronts at the end of the channel (Fig 3C and 3E) was measured by counting front arrivals at the end of the channel of indicated length.

The frequency of fronts reaching a certain distance (Fig 3D) was measured by counting peaks of activity along the temporal axis at the given distance from the beginning of the channel, in a channel of length $L = 1000$. $N = 30$ simulations with 500 fronts each were performed for each initial inter-front interval and (for Fig 3C and 3E) channel length.

Information transmission rate – numerical estimation. To estimate the information transmission rate (Figs 4, and 6D; Fig G, H, panel b, and I in S1 Appendix), $N = 100$ random sequences of 500 binary symbols S were generated, determining, for each slot, whether a front should be initiated. Unless stated otherwise, the probability of initiating a front was $\frac{1}{2}$. For each channel width, the average inverse front velocity was estimated numerically as described above (based on $N = 3000$ simulations) and used to predict the expected front transit time. For each front sequence, simulations were performed and, for each slot (with associated time t_{slot}), we computed the expected arrival time $t_{\text{expected}} = t_{\text{slot}} + \langle \tau_{\text{transit}} \rangle = t_{\text{slot}} + L \langle v^{-1} \rangle$ and recorded the difference $\Delta t = t_{\text{arrival}} - t_{\text{expected}}$ between the closest arrival time (t_{arrival}) and t_{expected} . Note that we computed t_{expected} and Δt for each slot, regardless of whether a front was initiated in it or not. In Fig E in S1 Appendix we show histograms of Δt for $S = 1$ and $S = 0$.

Next, we calculated the mutual information per slot MI_{slot} as information between the binary symbol S and variable Δt as follows. The differences Δt , binned with 1-minute resolution, were counted across all slots and stimulation sequences. Conditional entropy $H(S | \Delta t)$ was computed using a k NN-based algorithm with Miller–Madow bias correction [30,31]. In short: if a particular difference $\Delta t'$ occurred $n \geq k = 25$ times, numbers of occurrences originating from slots with $S = 0$ and $S = 1$ were counted (n_0 and n_1 , respectively) and the following formula was used for conditional entropy estimation:

$$H(S | \Delta t = \Delta t') = \frac{n_0}{n} \log_2 \frac{n_0}{n} + \frac{n_1}{n} \log_2 \frac{n_1}{n} + MM, \tag{7}$$

where $MM = 1/(2n \ln 2)$ if $n_0 \neq 0$ and $n_1 \neq 0$, and $MM = 0$ otherwise. If $n < k$, in order to ensure proper sample size, the calculation of n_0 , n_1 and n was repeated including not only points with $\Delta t = \Delta t'$, but also with Δt closest to $\Delta t'$ so that k data points were used in total.

The conditional entropy $H(S | \Delta t = \Delta t')$ was averaged across all data points to obtain $H(S | \Delta t)$, and subtracted from $H(S) = 1$ bit to obtain MI_{slot} according to the formula

$$MI_{\text{slot}} = I(S; \Delta t) = H(S) - H(S | \Delta t). \tag{8}$$

In cases where the MI_{slot} estimation was based on more than one front (Text A and Fig I in S1 Appendix), a vector of arrival time differences with respect to the expected arrival time was used in place of Δt ; Euclidean norm was used to find the nearest neighbors in the case of $n < k$.

Bitrate optimization was conducted by scanning the parameter space around a value predicted by earlier results (Figs 4E, 4F, 6D, and Fig G in S1 Appendix) or by a custom algorithm (Fig 4C and 4D).

Information transmission rate – semi-analytical predictions. To predict the information transmission rate for distant fronts (Fig 4B), we used the coefficients a_{spawn} , a_{fail} , W_{spawn} , and W_{fail} obtained based on single-front simulations (Fig 2C; Eqs (1–2)) to calculate λ_{spawn} and λ_{fail} . Then, we computed the probability that a front in a front train is eliminated

$$p = \exp\left(-L \times (\lambda_{\text{fail}} + \gamma \times \lambda_{\text{spawn}} \times n_{\text{backward}})\right), \tag{9}$$

where $n_{\text{backward}} = 1.285$ is the expected number of backward fronts spawned in a single event (obtained from data shown in Fig 2D) and γ is the probability that a backward front collides with a next front before reaching the channel beginning. We used $\gamma = 1$ to obtain the

distant-front value shown in Fig 4B. Based on the probability of front extinction p we constructed the confusion matrix

$$\begin{array}{cc|cc} \text{sent} \backslash \text{received} & 0 & 1 & \\ \hline 0 & 1-q & 0 & \\ 1 & qp & q(1-p) & \end{array}, \quad (10)$$

where q is the probability of sending '1' in the binary protocol. Then we computed MI_{slot} using the standard formula

$$MI_{\text{slot}} = \sum_{i,j} c_{ij} (\log_2 c_{ij} - \log_2 \sum_i c_{i,j} - \log_2 \sum_j c_{i,j}) \quad (11)$$

where c_{ij} are the entries of the confusion matrix, obtaining

$$MI_{\text{slot}} = -q \log_2 q + (1-q+pq) \log_2 (1-q+pq) + pq \log_2 (pq), \quad (12)$$

which in the case of $q = \frac{1}{2}$ simplifies to

$$MI_{\text{slot}} = 1 - \frac{1}{2} ((p+1) \log_2 (p+1) - p \log_2 p). \quad (13)$$

Eventually, we computed the information transmission rate as

$$r = \frac{MI_{\text{slot}}}{T_{\text{slot}}}. \quad (14)$$

To include the effect of interaction between fronts (Fig 4G–4I, dotted line), we took into account the probabilities of disruptive events $p_{\text{fail}} = p_{\text{propagation failure}} + p_{\text{immediate failure}}$ and p_{spawn} obtained from the numerical simulations with two fronts at different intervals (Fig 3B), as a function of the initial interval. To account for the fact that in the binary protocol the initial interval between fronts can be any multiplicity of the inter-slot interval T_{slot} (interval of length $k \times T_{\text{slot}}$ has probability $q(1-q)^{k-1}$), we averaged the probabilities p_{fail} and p_{spawn} using the formula

$$p_{\text{event}} = \sum_{k=1}^{\infty} q(1-q)^{k-1} \times p_{\text{event}}(k \times T_{\text{slot}}), \quad (15)$$

where p_{event} is either p_{fail} or p_{spawn} and $q = \frac{1}{2}$ is assumed. Then, we approximated the probability that a front is eliminated with the formula

$$p = 1 - \frac{1 - p_{\text{fail}}}{1 + \gamma \times p_{\text{spawn}} \times n_{\text{backward}}}, \quad (16)$$

where $n_{\text{backward}} = 1.285$ was taken from the single-front simulations and γ was calculated as

$$\gamma = \sum_{k=1}^{\infty} q(1-q)^{k-1} \times \max \left(0; 1 - k \times \frac{v T_{\text{slot}}}{2L} \right). \quad (17)$$

Based on p , we calculated MI_{slot} and bitrate using Eq (12) and Eq (14). Note that, unlike before, in this approach we used probabilities of disruptive events rather than propensities, which required running the two-front simulations for each channel length separately (analogous to Fig 3B, in which only results for $L = 300$ are presented). This was necessary, as the probabilities do not scale linearly with the channel length, due to the fact that the disruptive events are more likely to take place near the beginning of the channel.

To additionally include the effect of the transit time variance (Fig 4G–4I, dashed line), we assumed that the transit time has a normal distribution with $\sigma^2 = \sigma_{\text{transit}}^2 = \sigma_0^2 L$ and that a ‘1’ was received whenever a front arrived within $T_{\text{slot}}/2$ from t_{expected} (note that is *not* what we do in the numerical approach). The probability of a front being attributed to the correct slot, conditioned on that it reaches the channel end, is then $p_{\text{accurate}} = \text{erf}(T_{\text{slot}} / (2\sqrt{2} \sigma_0 \sqrt{L}))$. We modified the elimination probability $p' = p + (1-p)(1-p_{\text{accurate}})$ and computed the probability that a front was mistakenly detected in a slot in which no front was initiated $p_{\text{fake}} = q(1-p)(1-p_{\text{accurate}}) - 1/4 q^2 (1-p)^2 (1-p_{\text{accurate}})^2$ (the latter term prevents double counting of cases in which fronts from both the previous and the next slot are attributed to the considered slot). We created the confusion matrix:

sent \ received	0	1
0	$(1-q)(1-p_{\text{fake}})$	$(1-q)p_{\text{fake}}$
1	$qp'(1-p_{\text{fake}})$	$q[(1-p') + p'p_{\text{fake}}]$

(18)

and computed MI_{slot} using the formula (11).

Supporting information

S1 Appendix. Containing the following material : Fig A. Front propagation speed. Fig B. Chaotic front spawning. Fig C. Propensity of disruptive events for inert boundary conditions. Fig D. Front speed reduction due to the vicinity of a previous front. Fig E. Histogram of the temporal distance Δt from the expected arrival time to the nearest arrival time, conditioned on whether a front was initiated in the given slot. Fig F. Dependence of W_{fail} and W_{spawn} on model parameters. Fig G. Bitrate dependence on the number of E, I, and R substates. **Text A with Fig H.** Maximum bitrate for non-equiprobable binary symbols. **Text B with Fig I.** Alternative decoding: Bitrate estimation based on more than one front arrival time. (PDF)

S1 Video. Propagation failure. Simulation time-lapse related to Fig 2A. (MP4)

S2 Video. New front spawning. Simulation time-lapse related to Fig 2A. (MP4)

S3 Video. Front collision and annihilation. Simulation time-lapse related to Fig 2B. (MP4)

S4 Video. Chaotic front spawning. Simulation time-lapse related to Fig B in S1 Appendix (MP4)

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Writing – review & editing: Paweł Nałęcz-Jawecki, Frederic Grabowski, Marek Kochańczyk, Tomasz Lipniacki.

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Appendix: Supporting Information

Information transmission in a cell monolayer: A numerical study

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This file contains the following material:

- **Fig A. Front propagation speed.**
- **Fig B. Chaotic front spawning.**
- **Fig C. Propensity of disruptive events for inert boundary conditions.**
- **Fig D. Front speed reduction due to the vicinity of a previous front.**
- **Fig E. Histogram of the temporal distance Δt from the expected arrival time to the nearest arrival time, conditioned on whether a front was initiated in the given slot.**
- **Fig F. Dependence of W_{fail} and W_{spawn} on model parameters.**
- **Fig G. Bitrate dependence on the number of E, I, and R substates.**
- **Text A with Fig H. Maximum bitrate for non-equiprobable binary symbols.**
- **Text B with Fig I. Alternative decoding: Bitrate estimation based on more than one front arrival time.**

Software used for simulations is available on GitHub <https://github.com/kochanczyk/qeirq>.
Scripts used to create the figures together with generated data are available in a separate repository: <https://github.com/pSzyc/visavis-seir>.

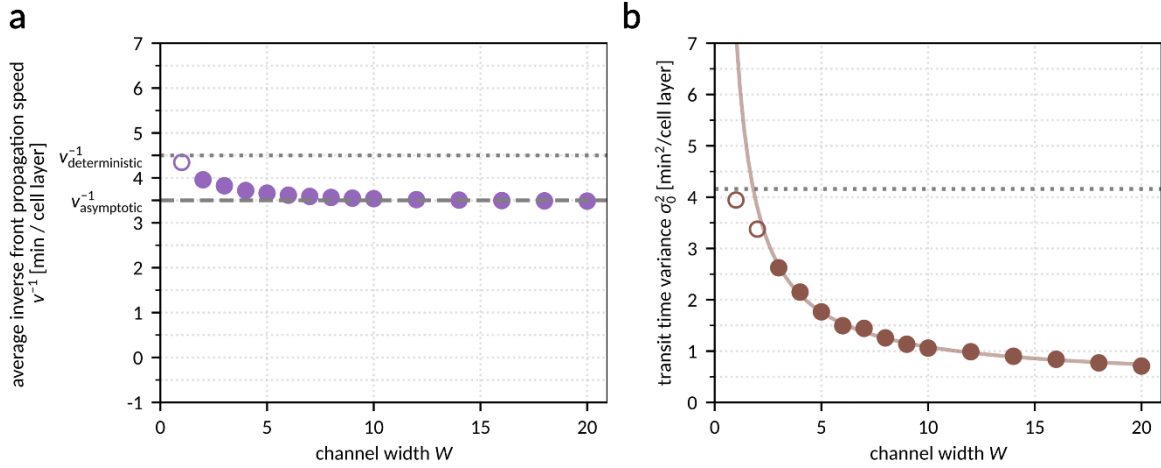


Fig A. Front propagation speed.

- a** Average inverse front speed as a function of channel width. Dotted line denotes the analytically computed velocity $v^{-1}_{\text{deterministic}} = 4.5$ min/cell layer, equal to $\langle v^{-1} \rangle$ for $W = 1$. Dashed line denotes the asymptotic inverse velocity of 3.5 min/cell layer.
- b** Traveling time variance normalized with the channel length, as a function of channel width. Dotted line denotes the analytically computed value $\sigma_{0,\text{analytical}}^2 \approx 4.16$ min²/cell layer for $W = 1$ in the stochastic model. Solid line is the function $\sigma_0^2 = a / W + b$ fitted to data for $W > 2$; best fit coefficients: $a \approx 6.8$ min²/layer², $b \approx 0.4$ min²/layer.

Data from 30,000 simulations in a channel of length $L = 300$ (filled circles) or $L = 30$ (empty circles).

chaotic front spawning

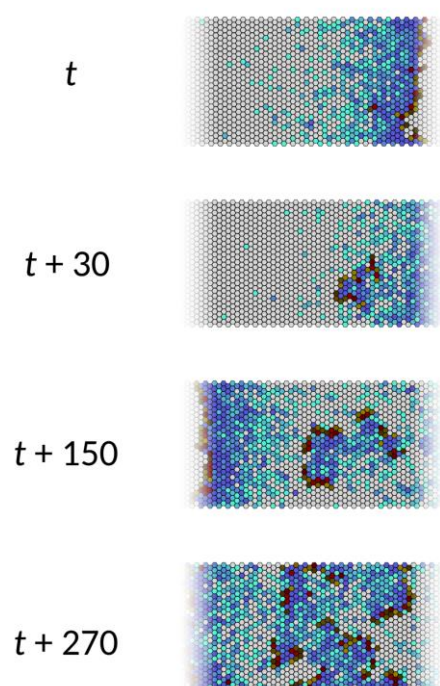


Fig B. Chaotic front spawning. Snapshots from simulations with fronts propagating in directions not parallel to the channel axis. See also S4 Video. Channel width $W = 24$.

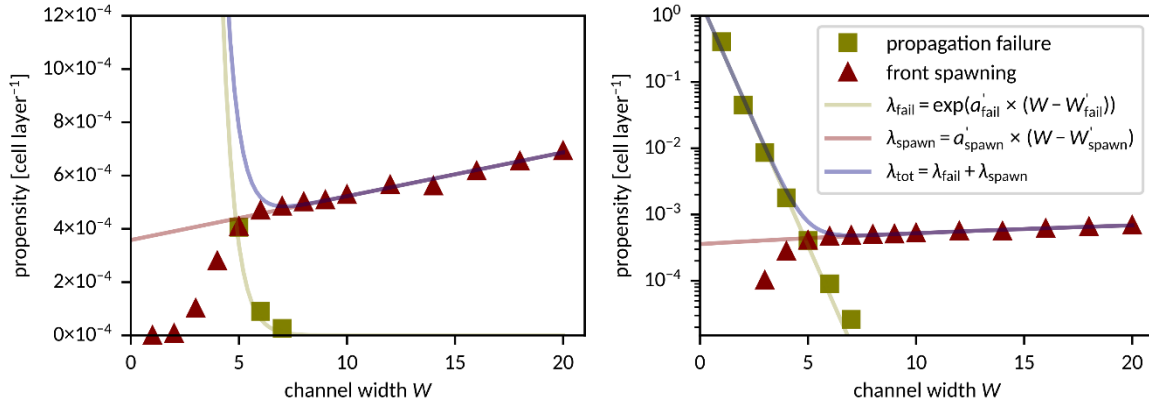


Fig C. Propensity of disruptive events for inert boundary conditions. Inert (non-periodic) boundary conditions along the longer edge are applied. The analysis of disruptive events associated with front propagation corresponds to that in main text Fig 2C.

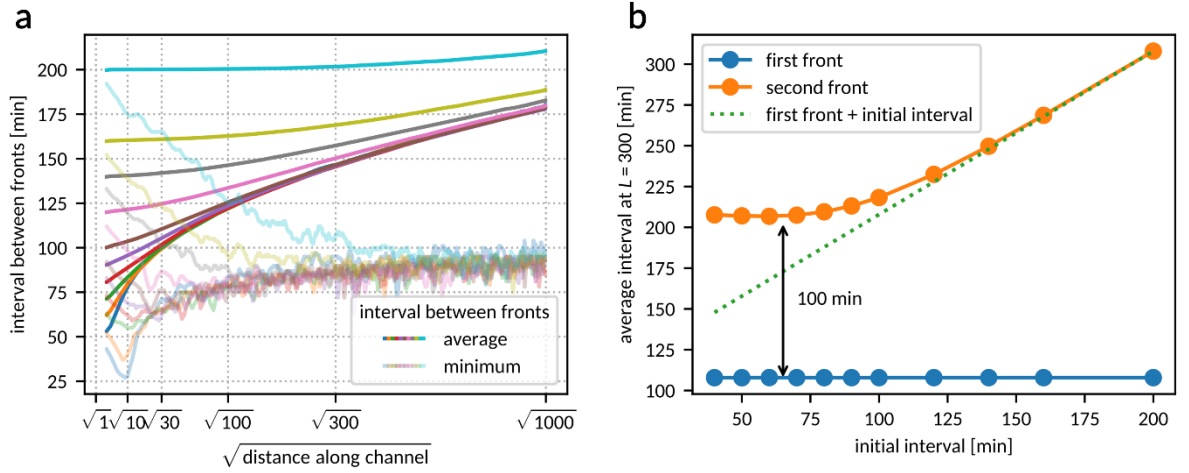


Fig D. Front speed reduction due to the vicinity of a previous front.

- a** The average (intense-colored curves) and minimal (pale-colored curves) time difference between the moments when two fronts reach a particular distance along the channel. Curves correspond to different initial intervals between the two fronts, ranging from 50 min to 200 min. $N = 30,000$ simulations per curve.
- b** Time when each of the two fronts reaches the end of the channel ($L = 30$) as a function of the initial interval.

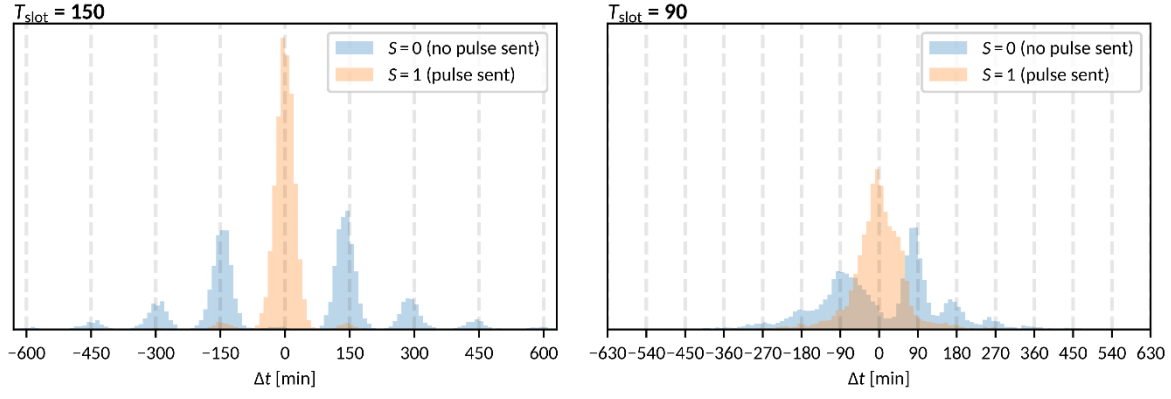


Fig E. Histogram of the temporal distance Δt from the expected arrival time to the nearest arrival time, conditioned on whether a front was initiated in the given slot. Conditional entropy $H(S | \Delta t)$ was used to estimate the amount of information lost during front propagation through the channel. Simulations as in Fig 4. Channel width $W = 6$, channel length $L = 300$ and two different inter-slot intervals were used. Kinetic parameters were set to the nominal values (Fig 1B).

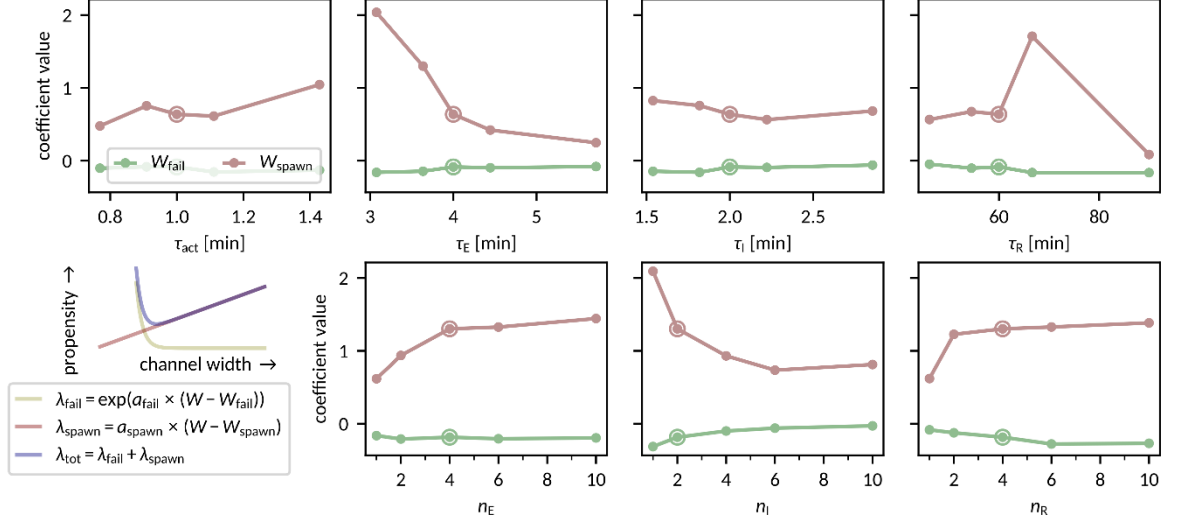


Fig F. Dependence of W_{fail} and W_{spawn} on model parameters. The encircled dots in each panel correspond to the nominal parameter set given in Fig 1B.

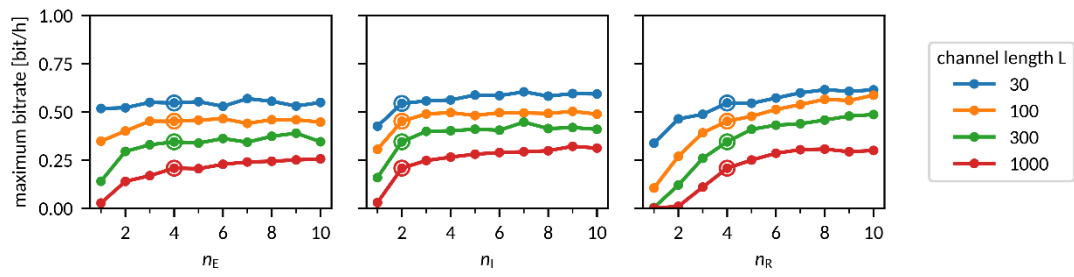


Fig G. Bitrate dependence on the number of E, I, and R substates. The encircled dots in each panel correspond to the nominal parameter set given in Fig 1B.

Text A

Maximum bitrate for non-equiprobable binary symbols.

Throughout the paper, we restricted ourselves to binary protocols with equiprobable ($q = \frac{1}{2}$) binary symbols $S \in \{0, 1\}$. Such protocols maximize the information transmission rate in the case when the confusion matrix is symmetric. In our case, the symmetricity of the confusion matrix is broken by the possibility of front extinction, due to which '0's are transmitted more reliably than '1's. In such a case the information transmission rate is maximized for $q < \frac{1}{2}$, however the difference with respect to $q = \frac{1}{2}$ is marginal, Fig H panel a.

In the case of short channels ($L = 30$), standard deviation of transit time σ_{transit} is small, and sent information is mostly lost due to front propagation failure. We may see that for $L = 30$ the substantially higher information transmission rate may be attained for $q \approx 0.2$ and $T_{\text{slot}} \approx 50$ min much shorter than refractory time $T_R \approx 96.6$ min, Fig H panel b. In this case, two following '1's cannot be transmitted, and the small value of q is beneficial as it implies low probability of sending two '1's in a row. Based on our previous study [1] we know that a protocol in which $\sigma_{\text{transit}} < T_{\text{slot}} < T_R$ but sending two '1's in a row is forbidden, results in even (somewhat) higher information transmission rate.

For longer channels the information transmission rate obtained for $q < \frac{1}{2}$ is only marginally higher than that obtained for $q = \frac{1}{2}$.

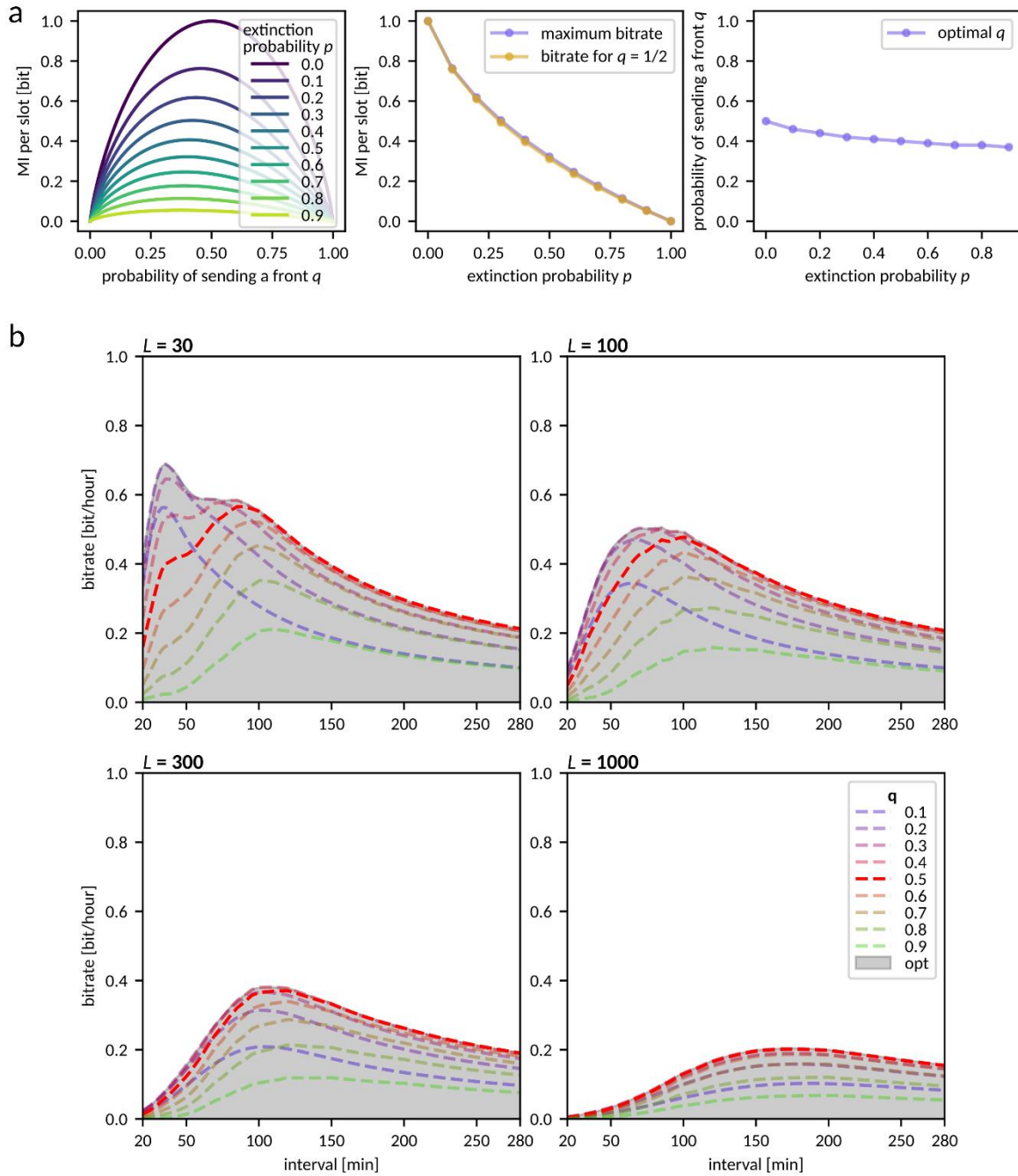


Fig H. Maximum bitrate for non-equiprobable binary symbols.

- a** Theoretical prediction of the bitrate in a binary protocol with fronts sent with probability q , assuming various probabilities of front extinction p .
- b** Measured bitrate for different front sending probabilities q as a function of T_{slot} . Shaded area depicts the bitrate for optimized q .

Text B

Alternative decoding: Bitrate estimation based on more than one front arrival time.

In the main text, the bitrate was estimated based on the arrival times of individual fronts. One could argue, however, that inference based on several consecutive fronts may yield higher bitrate estimates. Using more fronts could help discern whether a given received front originated from a true sent front or rather was spawned in a disruptive event. To verify the extent to which the information transmission rate can be improved in this way, we recomputed the bitrate estimates based on the arrival time of the front closest to t_{expected} and the arrival time of the preceding front (see Fig I below). While the bitrate increase is marginal for long inter-slot intervals, for intervals below T_R the information transmission rate estimate is noticeably higher, especially in short channels. For $L = 30$, where $\sigma_{\text{transit}} \approx 7$ min is much smaller than $T_R \approx 100$ min, a secondary local bitrate maximum appears close to $T_R/2$. In this regime, if two fronts are sent in subsequent slots, the latter will almost certainly be lost. However, this regime allows for time intervals of $3/2 T_R$, $5/2 T_R$, $7/2 T_R \dots$, which increases the input information rate. Decoding based on two arrival times helps to distinguish whether a front was not received when expected due to it not being sent or because the channel was blocked by a front sent in the previous slot. Taking more than two front arrivals into account does not bring any further benefit (see Fig I below).

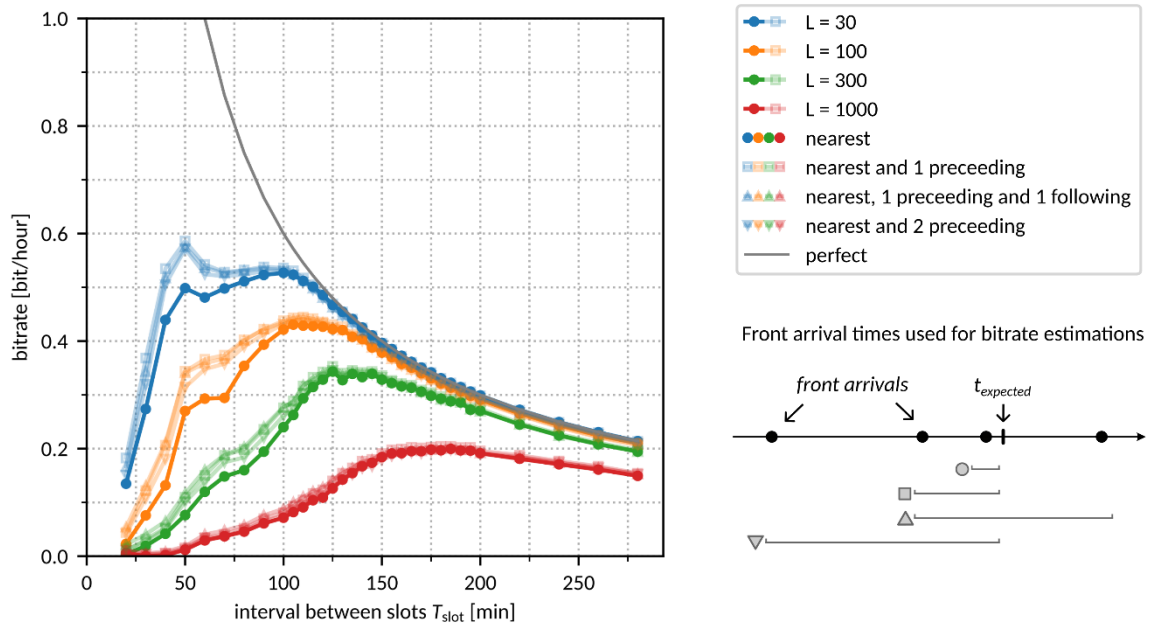


Fig I. Bitrate estimation based on more than one front arrival time. Figure corresponding to Fig 4A in the main text.

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Author Contribution Statements

Author contributions

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<https://doi.org/10.1371/journal.pcbi.1011155>

I declare that, as my contribution to the above article, I:

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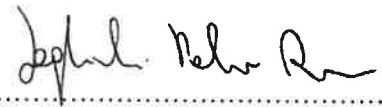
Author contributions

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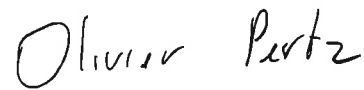
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- wrote the scripts and performed the analysis,
- prepared most of the figures and original manuscript.


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<https://doi.org/10.1038/s41598-025-23075-y>

I declare that, as my contribution to the above article, I:

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A handwritten signature in black ink, appearing to read 'Sunnie Li', is written over a horizontal dotted line.

Sunnie Li

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- conceived the study,
- prepared scripts for batch simulation and data analysis,
- designed and performed simulations, and analyzed the results,
- developed the numerical and semi-analytical methods for bitrate computation,
- prepared the figures and wrote the majority of the original manuscript.


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Author contributions

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I declare that, as my contribution to the above article, I:

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- contributed to figure preparation and revised the manuscript.

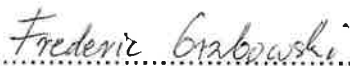

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Author contributions

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I declare that, as my contribution to the above article, I:

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I declare that, as my contribution to the above article, I:

- contributed software used for simulation,
- contributed to manuscript preparation and revision
- supervised the study.



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Marek Kochańczyk

Author contributions

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I declare that, as my contribution to the above article, I:

- conceived the study,
- supervised the study,
- revised the manuscript.



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Tomasz Lipniacki

