Modelling the molecular mechanisms of synaptic plasticity using systems biology approaches

Jeanette Hellgren Kotaleski and Kim T. Blackwell‡*

Abstract | Synaptic plasticity is thought to underlie learning and memory, but the complexity of the interactions between the ion channels, enzymes and genes that are involved in synaptic plasticity impedes a deep understanding of this phenomenon. Computer modelling has been used to investigate the information processing that is performed by the signalling pathways involved in synaptic plasticity in principal neurons of the hippocampus, striatum and cerebellum. In the past few years, new software developments that combine computational neuroscience techniques with systems biology techniques have allowed large-scale, kinetic models of the molecular mechanisms underlying long-term potentiation and long-term depression. We highlight important advancements produced by these quantitative modelling efforts and introduce promising approaches that use advancements in live-cell imaging.

Dynamics
Time-dependent changes in the activity or quantity of a variable.

Kinetics
The rates at which reactions or diffusion occur.

Systems biology
An inter-disciplinary field that focuses on the relationships and interactions between components of complex biological systems.

**Royal Institute of Technology, School of Computer Science and Communication, AlbaNova University Center, 106 91 Stockholm, Sweden.*
‡Molecular Neuroscience Department, Krasnow Institute of Advanced Studies, George Mason University, Fairfax, Virginia 22030, USA.
Correspndence to J.H.K.
e-mail: jeanette@nada.kth.se
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Neurons respond differentially to specific temporal and spatial patterns of inputs. This response specificity is not always programmed into neurons; rather, it can develop as the animal interacts with the environment and thus is involved in information storage and memory storage. The response properties of a neuron change through the plasticity of synapses and ion channels (see below), which is therefore proposed to underlie learning and memory. Consequently, to understand how neurons store information, we need to understand how spatiotemporal patterns of synaptic input, combined with postsynaptic neuronal activity, produce neuronal plasticity and how such plasticity changes neuronal activity patterns. Current research has revealed that numerous molecules and complex interactions between them underlie plasticity[1,2]. Therefore, simple conceptual models are insufficient to adequately explain the molecular processes underlying neuronal plasticity. Instead, the roles of dynamics and complicated, nonlinear molecule interactions require quantitative computational models for in-depth understanding.

Two aspects of neuronal plasticity are important for information processing: plasticity of intrinsic excitability[3] — that is, the change in ion channel properties; and synaptic plasticity — that is, the change in the strength of synapses between two neurons. Although more is known about the signalling pathways underlying synaptic plasticity, many of these pathways also underlie the plasticity of intrinsic excitability. The modelling approaches presented in this Review could therefore also be applied to intrinsic excitability.

Two crucial technological advancements have recently accelerated the development of signalling pathway models. One is live-cell imaging, which has provided essential constraints regarding the kinetics (rate constants) and spatial organization of signalling pathways. This information is required to address aspects of information processing such as synaptic specificity and spatiotemporal pattern discrimination. The second advancement is in computer hardware and architecture, which has been crucial for improving the temporal and spatial scale of synaptic plasticity models, because simulating large spatial structures for long durations with high resolution requires trillions of calculations.

In this Review, we first briefly summarize the current understanding of the postsynaptic molecular mechanisms underlying the plasticity of glutamatergic synapses — not only to show their complexity and the consequent need for integrative and dynamic techniques to better understand these mechanisms, but also to provide a framework for computational models of postsynaptic signalling pathways. We then explain some computational approaches that have been used in systems biology and are of particular relevance to modelling synaptic plasticity, and illustrate how these computational approaches have made important discoveries in, and

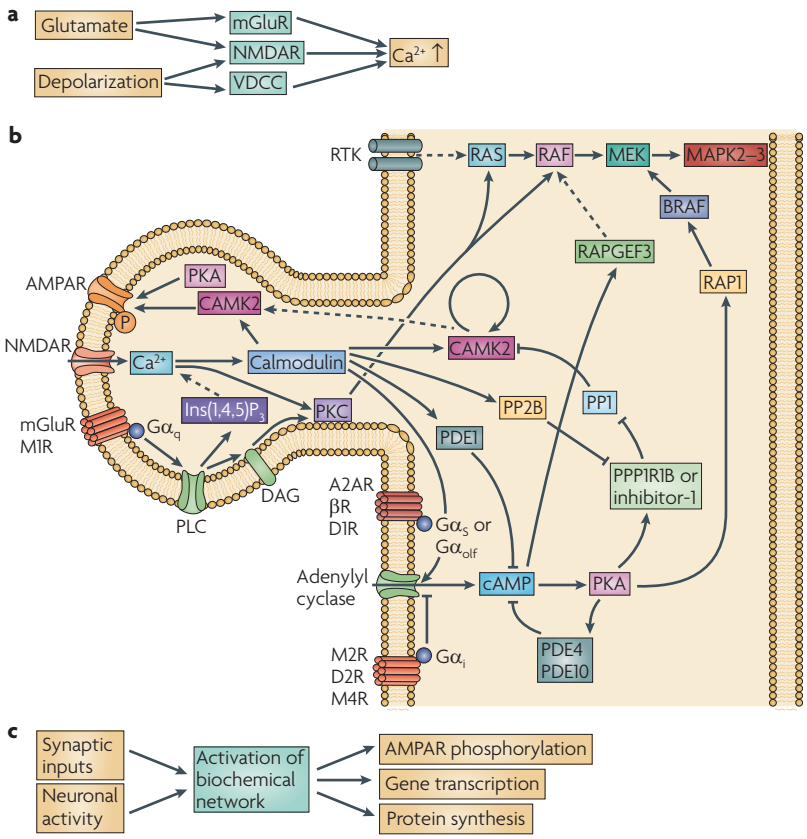


Figure 1 | **Signalling pathways underlying synaptic plasticity. a** | Presynaptic glutamate release and depolarization of the postsynaptic neuron leads to Ca^{2+} elevation in the postsynaptic cell. Glutamate is required for activation of NMDARs (*N*-methyl-D-aspartate receptors) and metabotropic glutamate receptors (mGluRs), and depolarization is required for activation of NMDARs[106] and voltage-dependent Ca^{2+} channels (VDCCs)[107]. The particular mechanism employed depends on the cell type. **b** | Signalling pathways leading to kinase activation and AMPAR (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor) phosphorylation. Only a subset of the known pathways is shown here, and not all of the pathways shown in this figure are involved in all neurons. Ca^{2+} activates Ca^{2+}–calmodulin-dependent protein kinase 2 (CAMK2), which phosphorylates the AMPAR GluR1 subunit, leading to increased numbers of functional AMPARs. CAMK2 can be persistently activated by autophosphorylation[108,109], which occurs when two adjacent subunits are bound to Ca^{2+}–calmodulin. This persistently active form of CAMK2 is most strongly implicated in hippocampal long-term potentiation (LTP). Dopamine D1 receptors (D1Rs), β-adrenergic receptors (βRs)[110,111] and the adenosine type 2A receptor (A2AR), coupled to the stimulatory G protein (G_s) or olfactory G protein (G_{olf}), contribute to LTP by activating adenylyl cyclase, whereas other dopamine D2 receptors (D2Rs) and muscarinic acetylcholine receptors (M2R and M4R) inhibit adenylyl cyclase. The cyclic AMP produced by adenylyl cyclase activates protein kinase A (PKA), which subsequently phosphorylates AMPAR GluR1 subunits and either protein phosphatase 1 regulatory subunit 1B (PPP1R1B; also known as DARPP32) or inhibitor-1 (REFS 112,113). These decrease phosphatase activity, allowing the persistence or enhancement of both AMPAR phosphorylation and insertion of AMPAR in the membrane. Some types of muscarinic acetylcholine receptors (M1Rs) and mGluRs are coupled to phospholipase C (PLC), which produces diacylglycerol (DAG) and inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$). Typical forms of PKC are activated by binding to both Ca^{2+} and DAG. MAPK2–3 (mitogen-activated protein kinase 2–3; also known as ERK1–ERK2) is activated through a pathway involving receptor tyrosine kinases (RTKs) via the RAS–RAF–MEK (MAPK–ERK kinase) pathway, and is necessary for the gene transcription and protein translation that underlies persistent forms of synaptic plasticity. In addition, MAPK2–3 can be indirectly activated by PKC, RAP guanine nucleotide exchange factor 3 (RAPGEF3; also known as EPAC), Ca^{2+} and PKA. **c** | For late-phase LTP and memory storage, a combination of synaptic inputs and neuronal activity leads to AMPAR phosphorylation and membrane insertion, gene transcription and protein translation. $G\alpha_i$, α-subunit of the inhibitory G protein; PDE, phosphodiesterase; PP, protein phosphatase .

contributions to, this field. Finally, we review some key computational models of the molecular mechanisms underlying synaptic plasticity that represent the diversity of simulation approaches, brain regions, molecular pathways and emergent information processing properties. Importantly, the models we describe were selected to highlight the discoveries that resulted from simulations, some of which have subsequently been confirmed in empirical experiments.

Molecular mechanisms of synaptic plasticity

The specific types of neuronal and synaptic activity that are required for the induction of long-term potentiation (LTP) and long-term depression (LTD) are diverse and depend on the brain region and cell type. Excellent reviews of these different forms of synaptic plasticity have been published elsewhere[4,5]. In general, the two main features of most induction protocols at excitatory, glutamatergic synapses are the presynaptic release of glutamate and postsynaptic depolarization[4], which together lead to an increase in the intracellular Ca^{2+} concentration in the postsynaptic cell through several mechanisms (FIG. 1a).

The increase in postsynaptic Ca^{2+} concentration, which is crucial for the induction of both LTP and LTD[6,7], leads to activation of many molecule species that are implicated in synaptic plasticity (FIG. 1b). In some systems, the magnitude of the Ca^{2+} elevation predicts whether an induction paradigm will produce potentiation or depression, with a large Ca^{2+} increase producing potentiation and a small increase producing depression[8,9]. Nonetheless, the Ca^{2+} concentration by itself is not always sufficient to predict the direction of plasticity[10,11]. In some cell types, the source of the Ca^{2+} influx influences whether LTD or LTP develops; for example, LTD requires activation of either metabotropic glutamate receptors (mGluRs) or L-type Ca^{2+} channels, whereas LTP is usually NMDA (*N*-methyl-D-aspartate) receptor dependent[5,12]. Furthermore, the nonlinear interactions between different sources of Ca^{2+} and its multiple target molecules make it difficult to predict the consequences of neural activity.

Several protein kinases and phosphatases, activated through transmembrane receptors, are implicated in either the induction or the maintenance of synaptic plasticity (FIG. 1b). Induction includes events during the stimulation protocol that lead to plasticity, whereas maintenance involves events that occur after plasticity has been induced. Maintenance events can be blocked by the application of drugs tens of minutes after induction. Ca^{2+}–calmodulin-dependent protein kinase 2 (CAMK2) — activated by Ca^{2+}-bound calmodulin — is required for hippocampal and neocortical LTP. Protein kinase A (PKA) is required for the induction of LTP in the striatum, and for the induction of a long-lasting form of NMDA-dependent LTP in the hippocampus (known as late-phase LTP)[13]. Protein kinase C (PKC) is required for the induction of LTD in the cerebellum and of mGluR-dependent LTP in the hippocampus[5]. In addition, atypical forms of PKC, such as protein kinase Mζ (PKMζ), although not required for induction, have a role in the

Box 1 | **A brief history of computational neuroscience**

One of the earliest and most well-known discoveries in computational neuroscience is the Hodgkin–Huxley model of the squid action potential[99]. Hodgkin and Huxley developed equations to describe the voltage clamp currents underlying the action potential. When these equations were simulated in current clamp mode, they reproduced the action potential. The computational aspect of this study made two important contributions. First, it revealed that the Na^+ and K^+ currents are sufficient to generate the action potential — the simulation showed that no additional current was needed to explain characteristics of action potentials such as the threshold, the refractory period and anode break (the occurrence of an action potential following release from hyperpolarization). Second, the gating mechanisms predicted by the equation were subsequently confirmed experimentally. Although single-channel recordings have shown that Markov kinetic models more accurately describe channel mechanisms, Hodgkin and Huxley's formulation nonetheless provided an efficient and parsimonious explanation for many aspects of ion channels and their contributions to neuronal activity.

The second major advance in computational neuroscience was the application and extension of the cable equation to dendrites[100]. Rall was the first to introduce a spatial dimension to neuronal signal processing and showed that the neuronal membrane has properties of a low-pass filter owing to its parallel resistive and capacitive characteristics. Parsimoniously, these same membrane properties cause attenuation and delay of synaptic potentials that are passively propagated along a dendrite. When synapses are represented as changes in membrane conductance, the cable equation can explain spatial and temporal summation of synaptic inputs. Although many dendrites have voltage-dependent ion channels, multi-compartmental resistive–capacitive models of neuronal morphology are the basis of more complicated neuronal models. These models are used to test the role of particular ion channels or neuronal morphology in neuronal activity.

Current clamp mode
Used for measuring membrane potential in response to current injection.

Markov kinetic model
A description of ion channel gating in which the change in channel conformation from one state to another depends only on the present state and not on previous states.

Cable equation
Describes the flow of electrical current along a wire, dendrite or axon.

Low-pass filter
A filter that reduces the amplitude of high-frequency signals while leaving low-frequency signals unaltered.

Resistive–capacitive models
Modelling a small neuronal compartment as a resistive element in parallel with a capacitor, and modelling dendrites as a connected set of these elements.

Network or graph theory
The study of the interconnectedness of related items. The nodes or vertices of a graph are individual items, and two nodes are linked with an edge if they influence each other.

maintenance of LTP[14,15]. Another kinase that is implicated in forms of synaptic plasticity that require transcription and translation is mitogen-activated protein kinase 2 (MAPK2; also known as ERK2)[16,17]. Inhibition of MAPK2 impairs some forms of LTP[18]; conversely, levels of MAPK2 are increased following some types of LTP[19].

Numerous experiments have elucidated the signalling pathways and neuromodulators that influence kinase activation, but the interactions of these many pathways at many points are nonlinear and therefore difficult to comprehend. For example, Ca^{2+}–calmodulin activates CAMK2, but in the hippocampus it also activates adenylyl cyclase types 1 and 8, leading to the production of cyclic AMP, and it activates phosphodiesterase type 1B, which degrades cAMP. Quantitative, dynamic computational modelling of signalling pathways can be used to investigate these complex interactions and predict important molecular mechanisms, which can guide investigators towards the most valuable experiments.

Computational modelling in neuroscience

Computational neuroscience has made tremendous contributions to the understanding of the electrical properties of neurons (BOX 1). Well-established methods for modelling ion channels and neuron morphology have made it possible to simulate the activity of identified ion channels, neuron morphology and, more recently, signalling pathways. Such models make explicit the assumptions about ion channel and reaction kinetics that are implicit in conceptual models. In general, these computational models address questions such as whether the known kinetics support the input–output relationships that are predicted by conceptual models, and whether particular ion channels or signalling pathways that are not included in conceptual models of a system, but are present in the tissue, are crucial for the functional aspects of that system.

Synaptic plasticity has been more difficult to capture in computational models. Unlike models for ion channels and neuron morphology, models of synaptic function do not simultaneously describe molecular mechanisms and accurately capture the experimental results. Indeed, there are two types of synaptic plasticity models. First, phenomenological models[20–23] accurately describe the relationship between *in vitro* neural activity and the resulting synaptic plasticity. Although these models are valuable for investigating changes in the behaviour of neural networks that result from synaptic plasticity, they do not address the mechanisms underlying plasticity. Second, models that are more mechanistic typically describe the role of Ca^{2+} in synaptic plasticity by combining traditional compartmental models that describe electrical activity[24–27] with equations that describe Ca^{2+} dynamics. These models have shown that the amplitude of the Ca^{2+} increase depends on the frequency of synaptic stimulation, owing to the voltage-dependence of the NMDA receptor, but they seldom include the kinases that are directly implicated in plasticity. The scarcity of complete models of synaptic plasticity reflects the complexity of the underlying mechanisms. Thus, a challenging and important goal for the future is to apply a systems level approach to achieve a deeper understanding of information processing and memory storage in neurons. Such systems level modelling is exceedingly difficult owing to the lack of information about the quantity and subcellular localization of key enzymes, but modelling can facilitate a thorough understanding of the many interacting, nonlinear processes in cells because of its integrative approach.

Computational approaches in systems biology

To gain an understanding of a biological system in terms of its function, regulation, adaptation and robustness, the individual components and the dynamic interactions between them over many temporal and spatial scales must first be understood. Systems biology aims to achieve this level of understanding by combining computational modelling and theoretical analysis with large-scale experimental investigation. Using this approach, fundamental questions — such as how complex cellular signalling networks are functionally organized — have been successfully addressed[28]. As the signalling cascades underlying synaptic plasticity are a subset of those studied by systems biologists, the approaches described in this section are applicable to computational studies of synaptic plasticity.

Graph theory and network topology. One approach in systems biology uses network or graph theory[29–31] to discover and predict the relationships between genes, proteins and other molecules. Combining experimental techniques with powerful computational and statistical

methods enables the identification of DNA sequences, proteins and lipids, and an assessment of whether such components can physically interact (or are otherwise associated in time or space)[32–35]. Graph theory takes advantage of, and indeed is required to make sense of, the data from the recently developed high-throughput methods that form the basis of fields such as genomics, proteomics and connectomics.

When graph theory is applied to biochemical networks, the nodes in the network represent molecular species and the edges represent the interactions between them. The rationale for this approach is that information about the topology that is inherent in those data can lead to the discovery of principles of network function and malfunction. This allows predictions to be made without knowing the systems dynamics. For example, using graph theory has led to the discovery that metabolic networks have properties of scale-free or small-world networks[36]. Unlike in randomly connected or highly connected networks, the path length between any two nodes in such networks is small, and the number of edges exhibits a power law. These properties imply that few proteins are highly connected and that many proteins have only a few interactions. Empirical experiments subsequently showed the importance of these highly connected proteins: they are conserved in evolution and are more likely to be essential than proteins with only a small number of connections[29]. Graph theory has also identified common mechanisms in several neurodegenerative diseases[37] and has predicted novel pathways, crucial molecules and the overall design logic in signalling networks that control, for example, the differentiation of the nervous system[38]. Information regarding alterations in protein interaction networks can also be used to predict and follow the clinical outcome in cancer treatments[39]. In systems biology studies of the brain, the phosphoprotein network of the synapse, which consists of more than 1,500 proteins, has been suggested as a starting point for linking molecules to behaviour[2,40]. The synaptic interaction networks might form the general basis for the evolutionary origin of behaviour and cognition[41].

Network motifs and network function. Several attempts have been made to identify functional network modules on the basis of network connectivity. The rationale is that, if larger networks are composed of smaller modules, then the computational function of the larger network can be understood from the computational function of the smaller modules[42]. Small recurring structural modules, called 'network motifs', are commonly found in biological systems[43]. Feedforward and feedback loops are examples of such motifs[31] (FIG. 2a).

One limitation of studying only the structure of motifs identified from network connectivity is that 'structure' cannot capture certain important dynamics. These include the time-dependent variations in membrane potential or phosphorylation state that are produced by synaptic inputs and action potentials and that depend on kinetics (for example, the rate of reactions). Such kinetic information is essential for determining the input–output relationship of motifs in various systems[44]. For example, consider a positive-feedback loop, in which protein A activates protein B, which further activates protein A. If the loop behaves as a switch with bistability (FIG. 2b,c), a sufficiently high input can change the output from an initial low-activation state to a high-activation state. In this case, the high activation is maintained even after the original input has been reduced. However, if the kinetics are slow, the loop will not change from one state to another, and there will be only a transient increase in output before the system reverts to the previous state. Dynamic behaviour of negative-feedback pathways (FIG. 2b) — whereby protein A activates protein B, which inhibits protein A in one or several intermediate steps — also depends on kinetics. If a feedback loop is fast — that is, with minimal delay — it will produce a general decrease in overall response. But if a substantial delay is incorporated into the feedback loop, or if the loop consists of several intermediate steps, a transient response or oscillations can occur[45,46]. These examples illustrate the need for models of dynamic activity, which require quantitative data to constrain the kinetics of the interactions to be modelled. Imaging techniques (BOX 2) can reveal the dynamics of crucial network components, even at the level of the single cell[47].

Dynamic modelling approaches. Dynamic modelling builds on the information about networks provided by graph theory, and evaluates time-dependent changes in the activity of proteins and other molecules. This approach is used to test hypotheses or to explore ideas *in silico* that are currently difficult or impossible to investigate experimentally. The simplest type of dynamic model, which does not require specific rate constants, is perhaps the Boolean logic network, in which quantities can have only two discrete values: 'present' and 'absent'. Logical operations (for example, 'protein A AND protein B are present') coupled with explicit time delays (for example, 'protein C is present later') can explain phenomena such as gene activation patterns[48]. A more mechanistic approach to understanding dynamics in gene, metabolic and signalling networks is to represent biochemical reactions using algebraic and ordinary differential equations[45,49]. Many of the parameters in such equations represent biochemically measurable values, such as enzyme turnover rates, affinities or on- and off-rates. Other parameters are slightly more abstract, such as those that represent an observed modulatory effect on a reaction. In both cases, many of the parameters are constrained by experimentally determined rate constants[50].

The family of MAPK pathways can be used to illustrate discoveries that were made using dynamic modelling approaches. The MAPK cascade consists of phosphorylation and dephosphorylation cycles (FIG. 2c). Many rounds of these cycles, with multi-site phosphorylations, amplify input signals considerably and can produce 'ultrasensitivity', such that a small change in the input near a threshold value[51] is translated into a sharp change in the output. Depending on the rate constants of the reactions and the relative abundance of the enzyme and the substrate, the MAPK cascade

Power law
The relationship between the dependent variable, y, and the independent variable, x, is described by $y = x^{\wedge(-k)}$.

Bistability
The existence of two stable or equilibrium states for the same input. Hysteresis is required for a system to achieve bistability.

Boolean logic network
A network with nodes that take one of two values — for example, activated or non-activated genes. Dynamics of the network are simulated using logical operations coupled with explicit time delay.

Exact stochastic simulation algorithm
An algorithm for the numerical solution of the state of a biochemical reaction system, which therefore produces a realistic actualization of the biochemical reactions.

can produce bistability[52], show oscillations[51] or show a graded response. Dynamic modelling explains how various network components affect the amplitude or temporal properties of MAPK activation, and this can control which of the various MAPK target molecules is activated[53,54]. Experiments have confirmed the role of these network components in controlling MAPK activation.

Dynamic stochastic approaches. A common but incorrect assumption in modelling is that the number of molecules in the studied system is always large enough to be represented as concentration. In fact, many subcellular compartments are small and can contain only limited numbers of molecules, and in larger compartments the concentration of a molecule can simply be low. For example, under resting conditions, a dendritic spine contains few Ca^{2+} molecules owing to its small volume. During stimulation, the number of Ca^{2+} molecules entering a spine is variable due to stochastic release of transmitter and gating of membrane channels, and the copy number of transcription factors in the nucleus is low. In all of these cases, stochastic fluctuations in the number of molecules add variability and can change the outcome of activating a signalling pathway. Clearly, stochastic effects should be taken into account when modelling a system.

Stochastic variability (also known as 'noise') can produce erroneous activity and eliminate stability (and bistability). Many methods that are used for stochastic modelling of biochemical reactions are based on the exact stochastic simulation algorithm[55]. In all of these methods, including variations that improve the computational efficiency of this algorithm, molecules are represented as points rather than as three-dimensional

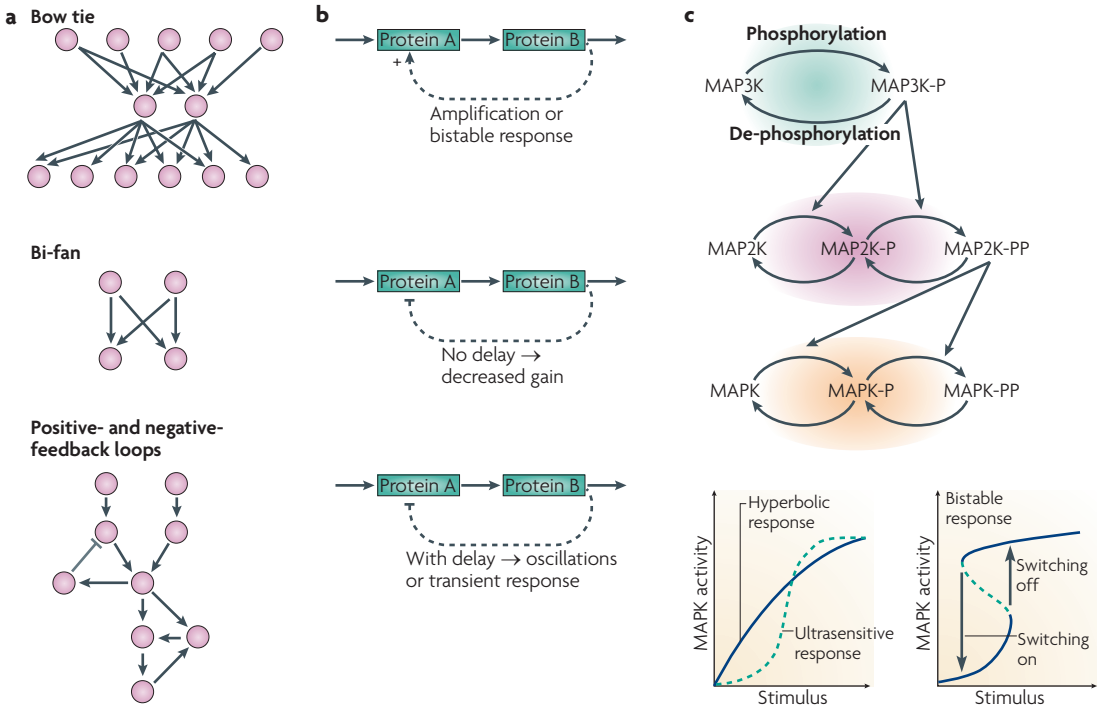


Figure 2 | **Signalling pathway motifs and computational units. a** | Examples of common structures found in cell signalling networks that have been identified using graph theory[31]. In the 'bow tie' structure (top), signals from many receptors converge onto a few cytosolic targets, which regulate many transcription factors. In the 'bi-fan' motif (middle), two input nodes directly cross-regulate target nodes. Negative-feedback loops (bottom) that include receptors usually occur close to the membrane, and positive-feedback loops (often highly nested) are more commonly found a few steps downstream of receptors. **b** | Dynamics in minimal pathway components in cell signalling networks. In a positive-feedback loop (top), the feedback results in a larger response than if no feedback were present. The interaction between voltage and L-type or non-inactivating inward Ca^{2+} channels shows this type of positive feedback. In some cases, positive feedback might result in bistability. In a negative-feedback loop with no (or in practical cases very little) delay (middle), the feedback results in a smaller response than if no feedback were present. Non-inactivating outward K^+ channels exhibit negative feedback. In a negative-feedback loop with delay (bottom), the delayed feedback reduces the response after an initial transient increase in response. Oscillations can occur in response to sustained input. **c** | A generic mitogen-activated protein kinase (MAPK) pathway. A single phosphorylation–dephosphorylation cycle is shown at the top. The MAPK cascade consists of three layers of such cycles, and the two lower layers have dual phosphorylation sites. Phosphorylation–dephosphorylation cycles can in certain conditions show 'ultrasensitivity' and behave like a threshold device (left graph). The layered cascade, with dual phosphorylation sites, can also sustain bistability (right graph). Bistability requires hysteresis: the response to an increasing input differs from the response to a decreasing input. By contrast, ultrasensitive systems exhibit a similar steep increase in output with small changes in input, but the output as a function of input is identical for increasing and decreasing inputs. MAP2K, MAPK kinase; MAP3K, MAPK kinase kinase.

Stochastic simulations
Simulations that use random-number generators — that is, Monte Carlo methods that are governed by rules of chance.

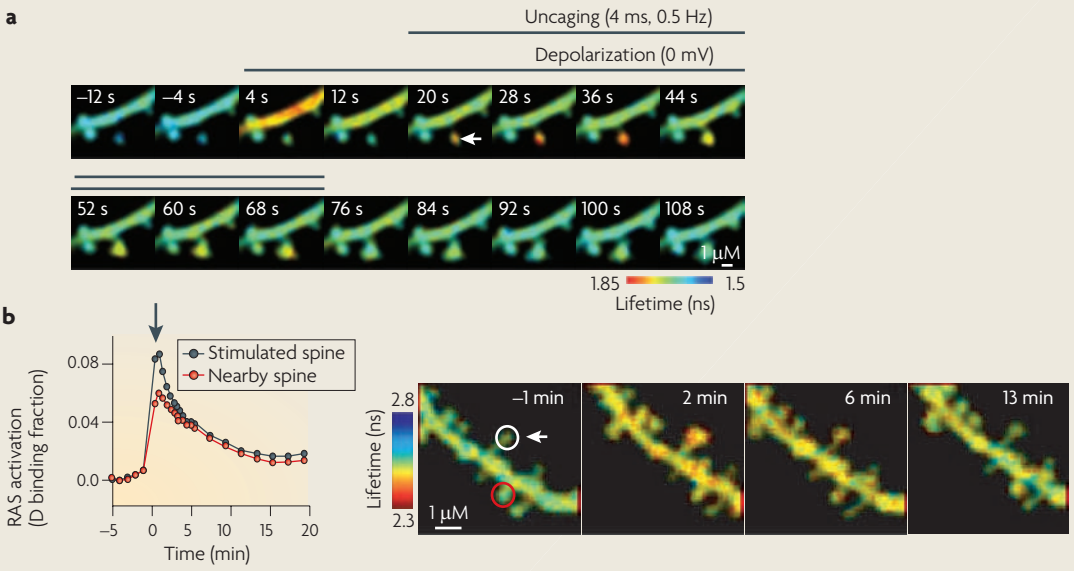
Deterministic model
A model that calculates quantities using differential equations and algebraic equations.

Glutamate uncaging
The process by which chemically caged glutamate is released by focal light. It is used to study the effects of postsynaptic activation with high temporal and spatial control.

objects, which ignores the fact that reactions are dependent on molecule collisions. Other methods for modelling stochastic reactions take geometry and charge of molecules into account, but such molecular dynamics simulations typically investigate single, bimolecular reactions and are rarely applied to large systems of reactions[56]. Stochastic simulations have revealed that signalling pathways such as positive-feedback loops, which behave as bistable switches in deterministic models, may no longer be bistable. Specifically, if the dynamics of the switch are fast, low levels of noise can activate the switch; and when the dynamics of the switch are adjusted so that it can resist effects of noise, the switch is not very sensitive to the signal. Stochastic modelling suggests that biological systems can reduce the effect of noise through coupled fast and slow feedback loops. Such loops ensure high sensitivity to the signal and reduce sensitivity to noise — both internal noise due to low numbers of molecules and external noise that is present in the input[57,58].

Dynamic spatial approaches. Another simplifying, but false, assumption in modelling is that biological systems are well mixed — that is, the molecules are equally distributed throughout the system so that there are no subcompartments. In reality, a signalling cascade might be activated at the plasma membrane where the receptors are located, and then cause activation of targets in the cell nucleus. For example, in the MAPK pathway, MAPK kinase kinase is phosphorylated in the membrane region and MAPK can have a nuclear target. Moreover, the kinases and phosphatases that control the activity of a particular enzyme might be located in different subcellular compartments, such as the membrane versus

Box 2 | **Spatial and temporal information provided by fluorescent-imaging techniques**



New techniques in imaging have provided high-resolution data in both the spatial and temporal domains to improve the reliability of model development. Several techniques have been developed using the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria*[101]. The GFP molecule has been modified to a range of colours which are excited by and emit at different wavelengths. Fluorescence resonance energy transfer (FRET)[102] is a technique in which the light emitted by cyan fluorescent protein (CFP) excites a yellow fluorescent protein (YFP) that functions as an acceptor when the two are in close proximity. CFP and YFP are attached either to two parts of a molecule (unimolecular FRET sensor) that changes conformation on ligand binding, or to two molecules that interact (bimolecular FRET sensor). The ratio of cyan to yellow emission represents the extent of ligand binding or interaction as a function of space and time. In fluorescence lifetime imaging microscopy (FLIM), interactions are measured by comparing the time (that is, lifetime) for one isolated fluorophore to emit light and return to the ground state, with a (shorter) time of the fluorescent event in the presence of an acceptor molecule in the environment. Fluorescence recovery after bleaching (FRAP) provides information on the rate of diffusion and the fraction of immobile versus mobile molecules. The GFP molecules in a small region are bleached, and the recovery results from unbleached molecules diffusing back into the region. Newer techniques are able to measure multiple molecules simultaneously[103]. These imaging techniques allow for visualization of fast events, such as Ca^{2+}, cyclic AMP or kinase activity transients. The figure illustrates FLIM imaging of hippocampal neurons following long-term potentiation induction protocols using glutamate uncaging (the white arrow in the figure indicates the uncaging site). 'Warmer' colours indicate shorter lifetimes and higher levels of activation. Active Ca^{2+}–calmodulin-dependent protein kinase 2 remains in an individually activated spine[104] (see the figure, part **a**) whereas Ca^{2+}-dependent RAS activity diffuses a short distance to neighbouring spines[105] (see the figure, part **b**). These imaging data from living cells define the limits of biochemical rate constants and reveal the spatial extent of molecule activation, which can be correlated with measures of synaptic plasticity[105]. Part **a** is reproduced from REF. 104 © 2009 Nature Publishing Groups. All rights reserved. Part **b** is reproduced, with permission, from REF. 105 © 2008 American Association for the Advancement of Science.

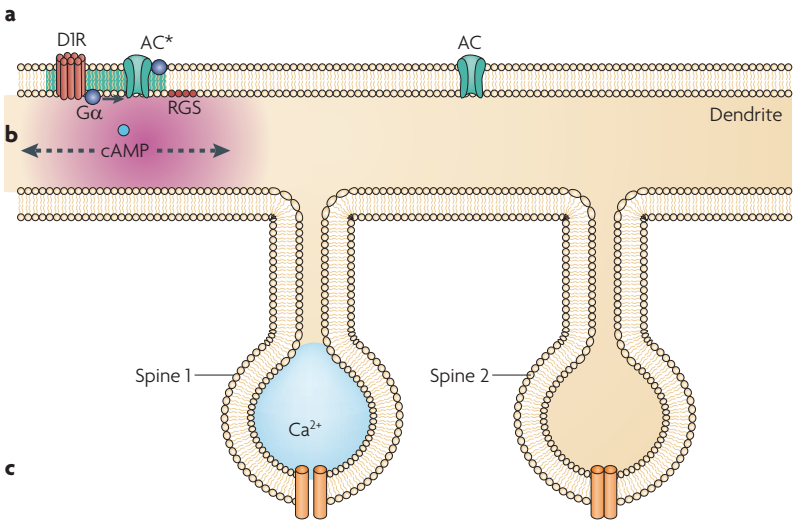


Figure 3 | **Spatial representation of a dendrite plus multiple dendritic spines are required to address input specificity and microdomains. a** | G proteins diffuse laterally within the membrane to activate adenylyl cyclase (AC). Limited mobility, coupled with regulators of G protein signalling (RGS) activity keeps AC activation (AC*) confined to a region near the receptor (green area), whereas AC further away remains inactive. **b** | Cyclic AMP diffuses rapidly within the cytosol; thus, the concentration gradient of cAMP is less steep than that of active AC, unless mechanisms to maintain the gradient are present, such as localized phosphodiesterases. **c** | Synaptic activation of spine 1, but not spine 2, results in an increase in Ca^{2+} concentration in spine 1 (blue area), which is kept limited to this spine by buffers and pumps and the narrow spine neck. Otherwise, diffusion would carry the Ca^{2+} message to spine 2, resulting in reduced synaptic specificity.

the cytosol or in a spine versus the dendrite (FIG. 3). Molecular interactions between such subcompartments depend on and can be constrained by the diffusion of molecules from one compartment to another — for example, when the rate of diffusion is slower than the rate of the reactions within subcompartments. Slow diffusion coupled with restricted localization or anchoring of proteins can thus result in microdomains of second messengers (FIG. 3).

Such reaction–diffusion problems can be modelled either deterministically or stochastically[59]. In deterministic methods, molecule concentrations are calculated as a function of space and time using partial differential equations. In stochastic approaches, molecule numbers are calculated as a function of space and time using Monte Carlo methods. Monte Carlo methods can be subdivided into methods that represent individual particles, each of which can reside at an arbitrary location[60], and those that divide space into discrete voxels[61]. Individual-particle methods have the advantage that they can take into account the size of the particles, which allows effects such as molecular crowding[62] to be simulated. Their disadvantage is that simulation of large numbers of molecules is still too expensive computationally. Some of the voxel-based methods allow several molecules to reside in each voxel (this is also known as the mesoscopic approach[63,64]) and monitor the number of each molecule species (or the number of molecules in different phosphorylation states). The advantage of these mesoscopic approaches is the ability to simulate large volumes and numbers of molecules; their disadvantage is that they cannot simulate particle crowding or the role of the location of a molecule within the postsynaptic density. For all these methods, a major challenge is how to model reactions involving multimeric molecules that have many phosphorylation states.

A voxel-based spatial stochastic model was used to demonstrate an unexpected role of regulators of G protein signalling (RGS) in the response properties of G protein-coupled receptors (GPCRs)[65]. RGS are important for deactivating GPCRs in many cell types, including neurons. The model helped to explain the mechanism that underlies a paradoxical experimental result, namely that the acceleration of GTP hydrolysis by RGS can enhance G protein activation. Model simulations showed that RGS reduce the depletion of Gα–GDP near the receptor, permitting rapid re-coupling of the receptor to G proteins for subsequent receptor activation. The model also showed that RGS helps spatial focusing of receptor activation: accelerated GTP hydrolysis reduces the diffusion of active G protein, limiting the spatial extent of GPCR activity. Similarly, phosphodiesterase 4 spatially focuses cAMP signalling by causing fast hydrolysis of cAMP, which increases the cAMP concentration at the membrane, near the adenylyl cyclase source[66,67].

Modelling molecular mechanisms of LTP and LTD

The past 10 years have seen a marked increase in the systems biology approach to studying synaptic plasticity. Not surprisingly, the models used to investigate synaptic plasticity involve similar techniques, approaches and scales as those used in systems biology. The computational approaches most commonly apply deterministic equations[68,69], although stochastic simulations have also been used more recently[70,71]. As in systems biology models, many of the computational models for synaptic plasticity involve one or a few spatial compartments. However, an increasing number of models include diffusion and an extended spatial scale that more accurately represents the unique morphology of neurons[72–74]. All of the models discussed below focus on a subset of events involved in the induction of synaptic plasticity, regardless of whether the molecule activation persists after induction.

Role of Ca^{2+}-activated pathways in LTP. Several computational models have explored the mechanisms by which CAMK2 can be persistently activated on a timescale that is comparable to the duration of long-term synaptic plasticity. One set of models used a dynamic modelling approach in which biochemical reactions are represented using ordinary differential equations and algebraic equations. Differential equations described Ca^{2+} binding to calmodulin and its subsequent binding to calcineurin and CAMK2, whereas algebraic equations were used to model Ca^{2+} activation of PKA and autophosphorylation of CAMK2 (which depends on the probability that two adjacent (versus non-adjacent) CAMK2 subunits are bound to Ca^{2+}–calmodulin[75–77]). One of the main predictions from these models was that CAMK2 remains phosphorylated (and thus active) by anchoring to proteins in

Partial differential equation
An equation that describes how the change in one variable depends on several other variables such as time and space.

Molecular crowding
A phenomenon in which the volume occupied by molecules is so large that molecules cannot diffuse freely in the cytoplasm. Reactions are either impeded or enhanced depending on the proximity of reacting molecules.

the postsynaptic density, and therefore remains protected from phosphatases[76]. The existence of two equilibrium states for the CAMK2 molecule led to another prediction: that CAMK2 activity exhibits bistability[77].

Another set of models of CAMK2 phosphorylation used non-spatial stochastic methods that take into account the small size of the spine and the postsynaptic density. A spine contains small numbers of each molecule species, and so molecular reactions and movements occur with some random variation. Stochastic models of CAMK2 activation can address questions such as whether *in vitro* experimental observations[78] and results from deterministic models are valid in the presence of random fluctuations[70,79,80]. One stochastic model included Ca^{2+} influx through the NMDA receptor and showed that both Ca^{2+} influx and CAMK2 were highly sensitive to the frequency of input stimulation, indicating that this sensitivity of Ca^{2+} dynamics propagates downstream to CAMK2 (REF. 79). One of the most exciting predictions from a model that included protein turnover (by replacing phosphorylated with unphosphorylated CAMK2 subunits) has been that synaptic stimulation can produce reliable and persistent phosphorylation of CAMK2 (REF. 70). Nonetheless, this model does not explain the molecular mechanisms underlying induction of late-phase LTP, which requires both transcription and translation.

Signalling pathways implicated in synaptic plasticity. The importance of interactions between signalling pathways has been shown in computational models of cerebellar LTD, in which paired stimulation of climbing fibres (CFs) and parallel fibres (PFs) is required to produce LTD[81,82]. Several differential-equation models include PF stimulation-induced activation of mGluRs on the dendritic spines of Purkinje cells — leading to diacylglycerol production and PKC activation postsynaptically — and CF stimulation-induced activation of voltage-dependent Ca^{2+} channels on Purkinje cell dendrites[69,83]. One model also included a feedback loop between MAPK and PKC[83] as a mechanism for producing bistability in PKC and MAPK activation. Simulations confirmed that concurrent stimulation of CFs and PFs is required for persistent LTD[84]. A subsequent model[69], which did not include MAPK activation but did include the release of Ca^{2+} through inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$) receptor channels, investigated the intriguing question of whether the PKC activation is sensitive to the temporal interval between PF and CF stimulation. An emergent feature of this model was that such temporal sensitivity exists and matches that observed in experiments of classical conditioning — a cerebellum-dependent type of learning[85]. The model further predicted that the temporal sensitivity was conveyed by several molecules, including the $Ins(1,4,5)P_3$ receptor and PKC[69]. It is unclear whether these results are seen when processes in the spine are modelled stochastically and spatially separated from the dendrites.

Another region of the brain in which pairing of inputs is essential for learning is the striatum, which receives inputs from both the cortex and the substantia nigra. During reward learning tasks, the striatum receives cortical glutamatergic inputs, leading to increases in Ca^{2+} concentration paired with nigral dopaminergic inputs. A differential-equation model of signalling pathways activated by Ca^{2+} and dopamine and ultimately leading to phosphorylation of protein phosphatase 1 regulatory subunit 1B (PPP1R1B; also known as DARPP32)[68] reproduced many experimental measurements of PPP1R1B phosphorylation[86–88] on Thr34 and Thr75. The novel finding from this model was that the response of the modelled neuron to transient stimulation (which represents the cortical and nigral activity during reward learning in behavioural experiments) differs from the response to continuous stimulation: a prolonged Ca^{2+} elevation in the modelled neuron reduces, whereas a transient Ca^{2+} elevation enhances, phosphorylation of PPP1R1B on Thr34.

Both this and another differential-equation model[89] of striatal signalling pathways, which included phosphorylation of PPP1R1B on Ser102 and Ser137, further predicted that a large Ca^{2+}-dependent increase in the activity of protein phosphatase 2A (PP2A) mediated the observed Ca^{2+}-dependent decrease in phosphorylation of PPP1R1B at Thr75 (REF. 90). This Ca^{2+}-dependent activation of PP2A was subsequently tested experimentally[91]: the results indicated that, although PP2A activity was enhanced by Ca^{2+} increases, the enhancement was considerably less than what was predicted to account for the substantial dephosphorylation of Thr75. Thus, rather than simply confirming prior experiments, these models — combined with experimental investigations — led to the discovery that additional signalling pathways must be involved to explain the experimental results and thus that further investigation is needed.

A systems biology approach to synaptic plasticity. An alternative to including a subset of signalling pathways in a computational model (as in the models discussed above) is to include all the pathways that are known to be implicated in synaptic plasticity — including the MAPK, PKA, CAMK2 and PKC pathways — with the aim of identifying functional motifs such as feedback loops, gates and switches that operate in neurons[50]. Deterministic simulations[19,50,92] have revealed several emergent properties of the global network of interacting pathways that are not present in individual pathways. These emergent properties include ultrasensitivity, and sensitivity to patterns of synaptic input with specific temporal characteristics. One study integrated results from simulations and empirical experiments to investigate the mechanisms underlying the sensitivity of LTP to the temporal interval of synaptic stimulation. In this comprehensive study[19], the simulations showed that the level of MAPK phosphorylation depended on the time between stimulus trains; subsequent *in vitro* experiments confirmed the prediction that phosphorylation of MAPK had the same temporal sensitivity as LTP; further simulations showed that temporal sensitivity of PKMζ was an important contributing factor. The systems biology modelling approach illuminates the range

Stochastic resonance
A mechanism by which noise added to a system enhances the signal to noise ratio.

of computations that may be performed through intracellular signalling cascades, but it does not necessarily show which of the possible computations are being performed.

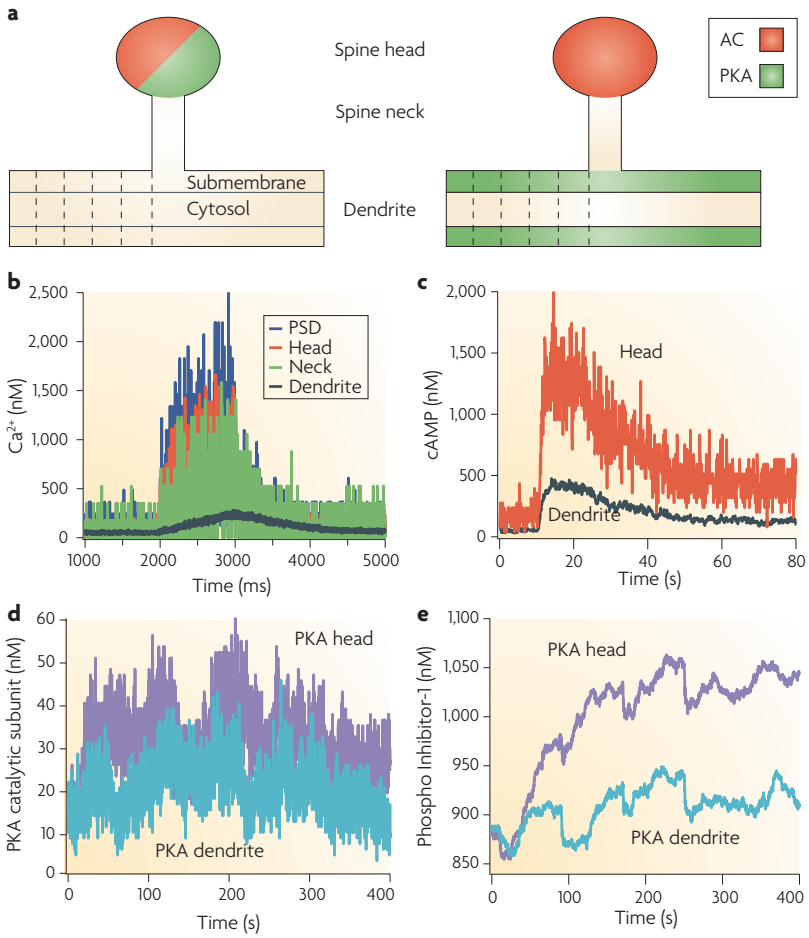
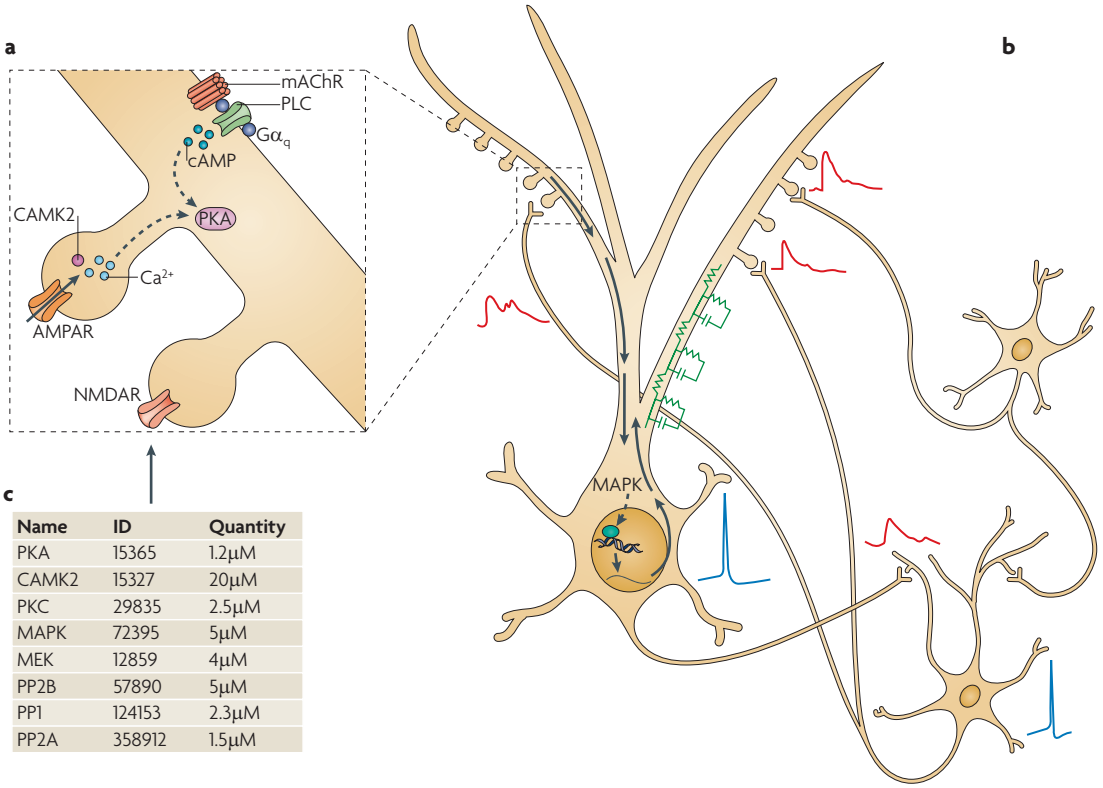


Figure 4 | **The role of proteins and spatial localization in long-term potentiation.** Spatial stochastic simulations using NeuroRD evaluate the role of anchoring proteins and microdomains in synaptic plasticity of hippocampal area CA1 (M. Kim, A. Chay and K.T.B., unpublished observations). **a** | NeuroRD is software for simulating stochastic reaction–diffusion systems in complex three-dimensional morphologies on a mesoscopic scale[67]. The software merges a mesoscopic diffusion algorithm[64] with elements of the tau-leap reaction algorithm[55]. The dendrite is subdivided into a submembrane region and a cytosolic region, and each region is further subdivided into voxels. Similarly, the spine is subdivided into head and neck voxels. The probability of reaction and diffusion are calculated from the voxel geometry and either the diffusion constant or the reaction constant. Given the reaction and diffusion probabilities, each time step involves generating the numbers of particles diffusing across each possible boundary and the number of reactions occurring, and updating the numbers of particles of each type in each voxel accordingly. The molecules and reactions in the system include the dopamine 1 receptor (D1R) and Ca^{2+}-activated pathways illustrated in FIG. 1B, but do not include mitogen-activated protein kinase pathways, or pathways coupled to the inhibitory G protein or $G\alpha_q$. In both cases, the D1Rs, G protein and adenylyl cylases (ACs) type 1 and type 8 are localized in the spine head. In one condition, protein kinase A (PKA) is colocalized with AC in the spine head (left). In the other condition, PKA is anchored to the dendrite submembrane (right). **b** | In both conditions, there is a Ca^{2+} gradient from spine to dendrite. **c** | AC produces cyclic AMP in the spine head (red trace), which diffuses into the dendrite (black trace), creating a gradient of cAMP. **d** | The gradient of cAMP leads to higher PKA activation when PKA is colocalized in the spine head (purple trace) than when PKA is in the dendrite (blue trace). **e** | The difference in PKA activation propagates downstream to yield greater phosphorylation of inhibitor-1 or protein phosphatase 1 (PP1) when PKA is in the spine (purple trace) compared with when it is in the dendrite (blue trace). PSD, postsynaptic density.

To compare simulated LTP to experimental LTP, computational models need to include AMPAR (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor) phosphorylation at the postsynaptic density, because the main read-out in plasticity experiments is the change in overall AMPAR conductance. Furthermore, they should use stochastic simulation techniques to take into account the small size of the postsynaptic density. Stochastic simulations[71,93] that included the same signalling pathways as those used in the deterministic models described above, as well as AMPAR phosphorylation, revealed that spontaneous transitions occur between states that were stable in the deterministic model. Thus, the system is not truly bistable, but rather exhibits a bi-modal distribution. These spontaneous transitions change thresholds into threshold ranges[71,93] as switches become either less sensitive to signals or more sensitive to noise. Similarly, the stochastic models showed that noise usually decreases the sensitivity to the temporal pattern of stimulation, but that stochastic resonance can enhance the response to particular temporal intervals. An important question addressed in this context is whether a synapse can exhibit potentiation, depression, de-potentiation or no change, depending on the stimulation pattern. Simulations of surface AMPAR expression have revealed that the phosphorylation of AMPARs by PKA together with AMPAR cycling in and out of the synapse produce bistability (in deterministic models) or a long time to spontaneously switch states (in stochastic models)[71] — similar to the bistability mechanism shown for MAPK. This mechanism is independent of the CAMK2 bistability, suggesting that synapses can have multiple stable states. Nonetheless, under conditions of few spontaneous state transitions, transient stimulation was unable to produce a state change, suggesting that additional mechanisms are required to explain the induction of LTP.

The dendrites of neurons permit important signal processing capabilities, such as the ability of individual dendrites to generate different electrical signals, which was shown by compartmental modelling of their electrical properties. Imaging studies (BOX 2) have revealed that these spatial characteristics of neurons, together with protein anchoring, result in microdomains of signalling molecules in neurons. Modelling this spatial aspect and evaluating the role of space is a relatively new approach to investigating neuronal plasticity[72,74] that has led to several fascinating discoveries. A deterministic spatial model of pathways involving cAMP (including those mediated by PKA, phosphodiesterase and MAPK) showed that neuronal morphology, in particular a high surface to volume ratio, is important but not sufficient for producing spatial microdomains[74]. Rather, both the biochemical network topology and kinetics are essential for the propagation of cAMP microdomains to downstream targets[74]. The most comprehensive deterministic spatial model of signalling pathways (including MAPK, PKA, CAMK2 and PKC) incorporated a multi-compartmental, multi-channel electrical model of a dendrite. Simulations of this model revealed that, unlike Ca^{2+} waves and action potentials, which propagate rapidly,



c

Name	ID	Quantity
PKA	15365	1.2μM
CAMK2	15327	20μM
PKC	29835	2.5μM
MAPK	72395	5μM
MEK	12859	4μM
PP2B	57890	5μM
PP1	124153	2.3μM
PP2A	358912	1.5μM

Figure 5 | **Computational neuroscience in the future integrates many scales and diverse modelling approaches.** Computational neuroscience in the future will integrate systems biology approaches to modelling the signalling pathways underlying synaptic plasticity with computational neuroscience approaches to modelling electrical activity in the neuron. **a** | Model neurons should incorporate data from experimental findings regarding the cellular and molecular events that result from neuronal activity. For example, both ionotropic receptors (for example, AMPARs (α-amino 3-hydroxyl 5-methyl 4-isoxazole-propionate receptors) and NMDARs (*N*-methyl-D-aspartate receptors)) and metabotropic receptors (for example, muscarinic acetylcholine receptors (mAChRs)) are activated by simulated synaptic inputs resulting from ongoing network activity. Membrane depolarization produces Ca^{2+} influx through voltage-dependent Ca^{2+} channels and NMDAR channels, and Ca^{2+}-dependent K^+ channels in turn modify membrane potential. Both Ca^{2+}-dependent and receptor-activated signalling pathways activate kinases (for example, protein kinase A (PKA)) and phosphatases, which may modify channel properties (not shown). **b** | Networks of such model neurons are required to understand the development of plasticity in response to realistic neuronal firing patterns, and to understand how the plasticity modifies network activity**.** Thus, many copies of the neuron model of electrical and chemical events will be connected to each other with synapses. **c** | Neuroinformatics databases and tools will play a key part in facilitating the development of large-scale data-driven models, ranging from molecules to network connections. The information in the databases ranges from neuron morphology to kinetics of signalling pathways and ion channels, and will be used to create more realistic neuron models. CAMK2, Ca^{2+}–calmodulin-dependent protein kinase 2; MAPK, mitogen-activated protein kinase; MEK, MAPK–ERK kinase; PP, protein phosphatase.

activation of MAPK throughout the dendrite (which has been observed experimentally) does not result from diffusion or wave-like propagation of feedback loops, but requires depolarization-producing Ca^{2+} influx at multiple points along the dendrite[72].

In summary, computational models of synaptic plasticity have addressed whether the molecules that have been identified as being involved in synaptic plasticity can account for experimental observations, and which molecular mechanisms underlie the observations. Modelling — both the process of model development and simulation experiments — integrated with imaging experiments can test whether our understanding of these mechanisms is complete or whether a molecule has a hitherto unknown function, by making explicit the assumptions that are implicit in conceptual models.

Future directions

Computational models of synaptic plasticity will expand beyond a subset of relevant pathways and limited spatial domains by integrating findings from imaging and cell biology. The recent development of new imaging techniques has provided high-resolution data, both in the spatial and in the temporal domain — for example, regarding protein mobility and the dynamics of interactions with other proteins —improving the reliability and accuracy of computational models. Recent developments in spatial stochastic simulation techniques[63,94,95] (FIG. 4) allow us to assess the role in synaptic plasticity of microdomains resulting from neuron morphology (spines) and anchoring proteins (such as A kinase anchoring proteins) within a model of the larger volume of a dendrite with multiple spines (FIG. 4). A spatial

stochastic model of intracellular spine Ca^{2+} dynamics showed that Ca^{2+} activation of calmodulin is sensitive to the temporal interval between presynaptic glutamate release and the postsynaptic action potential[96]. Despite these advancements in simulation techniques, the development of large-scale simulations is exceedingly difficult owing to the limited knowledge about concentrations of enzymes and their subcellular localization. Nonetheless, such large-scale simulations are useful for assessing whether a proposed biological mechanism is plausible, and for evaluating alternative hypotheses.

Modelling signalling pathways alone is not sufficient for understanding synaptic plasticity. Because of the complexity of the interactions between membrane potential, ion channels and Ca^{2+} dynamics, a blending of techniques[97] that permits integration of multi-compartmental models of electrical activity is essential. Mutual interactions between membrane potential and ion channels constitute a feedback loop, as do the interactions between ion channels and Ca^{2+} dynamics. Future models should include modulation of voltage-dependent channels, which is crucial for plasticity of intrinsic excitability and in turn controls synaptic plasticity through altering the membrane potential (FIG. 5).

Modelling approaches that span large temporal and spatial scales are also important for investigating gene expression and structural changes, both of which are required for memory storage and late-phase LTP and LTD. Recent models have begun to address the role of local protein synthesis in LTP[98]. Increasingly powerful, efficient and multi-scale computational algorithms are required to simulate the movement of molecules — such as transcription initiation factors, mRNA and proteins — over long distances. Thus, modelling gene expression and memory storage requires an integration of millisecond processes over long timescales (hours) and an integration of processes involving submicron compartments over large spatial scales (mm).

Such computational advancements will require corresponding advancements in neuroinformatics and bioinformatics techniques to extract and integrate quantitative and dynamic information, so that this information can be used in computational investigations. Of particular relevance are databases of signalling pathways, rate constants and protein quantities in different brain regions and cell types. Furthermore, the plasticity of synapses and ion channels interacts nonlinearly with neuronal network activity, which influences plasticity mechanisms (thereby forming additional feedback loops). Thus, the development of synaptic plasticity and its consequences for network activity and behaviour requires simulations of large-scale networks of such neurons. Computational models of this scope will have broad applications, such as in investigating the mechanisms underlying disorders that are characterized by memory impairments.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES

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FURTHER INFORMATION

Jeanette Hellgren Kotaleski's homepage: http://www.csc.kth.se/~jeanette/
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