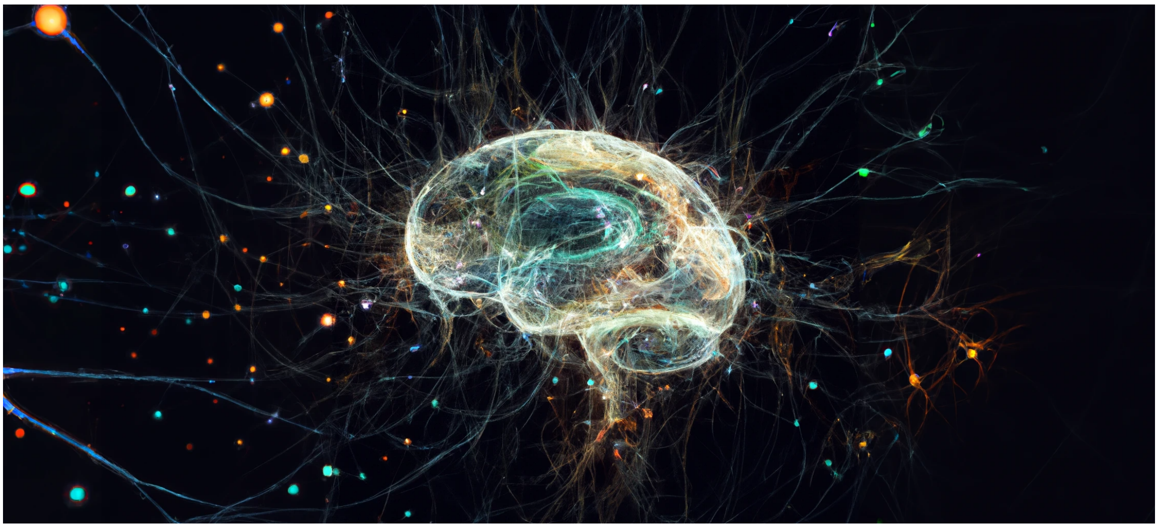
University of Liège
Department of Electrical Engineering & Computer Science

Uncovering the molecular and cellular mechanisms of reliable neuromodulation in highly heterogeneous neurons



a PhD dissertation summary

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Context and motivation

Brain activity is constantly shaped by the action of numerous neuromodulators and neuropeptides such as dopamine, serotonin, and histamine, to name a few. Neuromodulators dynamically influence single neuron activity and input/output properties as well as the strength and dynamics of synaptic connections, providing a mean to constantly adapt a neuronal network activity in response to ever changing needs, contexts and environments. To achieve this modulation, neuromodulators dynamically reshape the density, dynamics and kinetics of many single cell transmembrane proteins, making whole brain functional signaling strongly dependent on the robustness and reliability of neuromodulation mechanisms at the molecular and cellular levels.

Although neuromodulation has been shown to be ubiquitous in the nervous system for several decades, the mechanisms underlying their reliable action are still elusive to date for two main reasons. First, neuromodulators affect neuronal signaling through the activation of metabotropic receptors. These receptors do not directly influence membrane ion channels but involve second messengers that trigger complex signaling cascades. These signaling cascades eventually target ion channels in a neuron specific manner, resulting in a wide range of effects in different neuron types, most of which are very difficult to identify experimentally. Likewise, many different neuromodulators can target the same ion channels, or subset of ion channels, leading to possibly interfering effects. Secondly, past research has convincingly highlighted that ion channel densities and other properties, which are the end targets of neuromodulators, can be highly variable in neurons of the same types, sometimes varying up to 5 folds. This raises the question of how neuromodulators can possibly have reliable effects at the whole brain level while indirectly targeting such variable properties at the molecular and cellular levels.

Attempting to provide answers to this question is the core goal of this thesis. We propose to study the molecular and cellular mechanisms underlying reliable neuromodulation in highly variable neurons by combining approaches from experimental and computational neuroscience, dynamical systems and feedback control. Our innovative strategy will be to analyze cellular neuromodulation from a feedback systems perspective as a direct, feedforward neuromodulator action on ion channel density cannot lead to reliable effects on highly variable neurons. We will explore and quantify how various sets of ion channel properties can lead to similar firing activities, a property called degeneracy, by working with high dimensional conductance based models. We will exploit the qualitative physiological structure of metabotropic receptor signaling to construct a feedback control system capable of providing reliable neuromodulation in highly variable cells. To achieve this, newly defined functional variables will be linked to the intermediate signaling pathway by decomposing the neuromodulation process into three main steps: (i) the neuromodulator activates a metabotropic receptor, (ii) the receptor modifies the neuron activity target by acting on a second messenger (such as cyclic AMP), and (iii) the second messenger involves a feedback control loop to reach the novel activity target by tuning the properties of a predefined ion channel subset.

Keywords – Neuromodulation, Neuronal variability, Conductance-based model

Thesis summary

This thesis can be summarized in three main results, each flowing into the next.

Part 1: Functional Variable Identification and Degeneracy Mechanisms in Neuronal Activity

The initial phase of this thesis focuses on identifying functional variables that uniquely characterize neuronal activity and correlate with degenerate physiological variables (e.g., ion channel density). Establishing this set of variables is essential prior to designing a neuromodulation controller, as non-degenerate references and variables are required to resolve ambiguities stemming from neuronal parameter heterogeneity.

Through dimensionality reduction, specifically Principal Component Analysis (PCA), applied to populations of degenerate neurons, two physiological mechanisms underlying neuronal variability are revealed. These populations were generated by a random conductance search across two conductance-based models: one from the stomatogastric ganglion (STG) of the crab and the other from a dopaminergic neuron (DA). The post-processing step ensured a fixed phenotype across neurons, resulting in external homogeneity but internal heterogeneity, without assuming specific degeneracy mechanisms.

The first identified mechanism is homogeneous scaling of maximal ion channel conductances, where conductances are scaled by a common factor, determining the slope of a regression line. This is captured by the dominant PCA component in both models and can arise from homeostatic ion channel expression models, where the slope between conductances correlates with neuronal activity type. The second mechanism, captured by secondary PCA components, involves degeneracy in conductance ratios associated with neuronal dynamical properties. This cryptic variability only becomes apparent when perturbations, such as ion channel blockades, are introduced in neurons with variable conductance ratios.

Interestingly, these two mechanisms—homogeneous scaling and degenerate conductance ratios—may compete. Variability from both mechanisms leads to neuromodulation-dependent correlations or uncoupling of conductance values. When the manifolds of these mechanisms align, pairwise conductance correlations are high; when misaligned, correlations diminish. These correlations vary with the neuromodulated state. Under neuromodulation, homogeneous scaling rotates around the origin of parameter space, while variable conductance ratios translate within the space, leading to changes in alignment and corresponding shifts in correlation values (Fig 1).

This study suggests that neuromodulation of degenerate neuronal populations cannot be simplified to purely multiplicative or additive rules, as the underlying degeneracy emerges from these two distinct mathematical operations. However, the resulting neuromodulation rule is still straightforward but indirect. Neuromodulation follows a linear trajectory in the conductance space, though the path length varies between neurons. This indicates that the direction of neuromodulation is neuron-type-dependent, while the step length is determined by neuronal parameters. Furthermore, this work facilitates the development of algorithms capable of generating degenerate neuronal populations within seconds, a significant improvement over the random search techniques that require hours. The algorithm has also been adapted to modulate existing degenerate populations by computing new values for modulated conductances.

Part 2: Development of a GPCR-Based Molecular Controller for Reliable Neuromodulation

Building on the previously developed algorithms, a GPCR-based molecular controller was designed to achieve reliable neuromodulation in neuron models exhibiting high variability. The use of Dynamic Input Conductances (DICs) as control variables allowed the translation of a pre-defined neuromodulation rule into an online controller. This controller is capable of dynamically regulating neuronal activity in real time, using human-understandable references and neuromodulator concentrations as control benchmarks.

Treating neuronal excitability as a feedback control system addresses the inherent complexity of neuromodulation by consolidating the effects of multiple voltage-gated ion channels into a set of feedback gains operating on various timescales. In this framework, neuronal dynamics are modeled as a control system, where voltage-gated (and some calcium-gated) ion channels function as a controller that generates control signals, acting on a passive membrane that serves as the plant (Fig 2A). This feedback control perspective simplifies the investigation of neuromodulation by reducing the complexity of neuronal dynamics to a manageable system.

The primary targets of neuromodulators are ion channel densities, which determine the feedback gains of the controller on each timescale. Neuromodulation can therefore be viewed as an input to an adaptive control block, whose function is to fine-tune neuronal behavior by adjusting the feedback gains in a functionally relevant manner. The inputs to this adaptive control block correspond to neuromodulator concentration near the neuron and ion channel expression values. Biologically, these inputs could originate from an additional neuronal adaptive control mechanism, such as a homeostatic control block, which reads neuronal outputs (membrane potential and intracellular calcium concentration) and regulates ion channel expression to maintain neuronal excitability within safe physiological limits.

From this neuromodulation perspective, an adaptation mechanism was derived, as illustrated in Fig 2B. The proposed system consists of two main components: a reference generator block and a reference tracking block (feedforward and feedback, respectively). The outputs of this neuromodulation-dependent adaptive system are ion channel expression levels that modulate neuronal feedback gains.

The feedforward block represents the activation of a metabotropic receptor, which sets neuromodulation-dependent targets for neuronal excitability by generating a reference signal for modulated ion channel expression (blue block). A molecular network, activated by the metabotropic receptor, maps these target gains into specific references for ion channel expression. These reference expression levels are input into a proportional-integral (PI) regulated protein translation control system, which synthesizes new transmembrane proteins and facilitates their transport to the membrane through diffusion or active trafficking. Ion channel movement between intracellular and membrane compartments is modeled as passive diffusion, while ion channel turnover accounts for continuous degradation of transmembrane proteins in the intracellular domain (purple block). For each modulated conductance, a positive translation control signal, representing the synthesis of new transmembrane proteins, is computed by a classical negative feedback controller, which seeks to match the ion channel conductance to its reference value (green block).

The robustness of this adaptive gain control scheme was tested through simulations involving neurons with highly variable maximal conductance values, consistent with biological data. The results demonstrate that the proposed neuromodulation adaptive control system closely replicates the biological feedback mechanisms of neuromodulation. Despite significant variability in intrinsic ion channel conductances, the neuromodulated neurons exhibited minimal variability in their phenotypic properties.

Part 3: Interaction Between Neuromodulation and Homeostatic Regulation in Neurons and Robotics Applications

After validating the neuromodulation adaptive controller, we explored its interaction with a previously published model of homeostasis.

Neurons and circuits demonstrate an exceptional ability to regulate and maintain their electrical signaling properties despite various disturbances, such as protein turnover or external perturbations. This critical functionality is achieved through homeostatic regulation, exemplified by a model that maintains intracellular calcium levels by adjusting the conductances of all ion channels, either increasing or decreasing them, while preserving predefined correlations between conductances (O'Leary et al., 2014).

The two computational models—neuromodulation and homeostasis—offer contrasting regulatory approaches. Neuromodulation primarily alters the correlations between pairs of conductances within a subset of neuromodulated ion channels, impacting neuronal dynamics. In contrast, homeostatic regulation adjusts all conductances in the same direction to maintain a target calcium level, thus modifying passive neuronal properties while preserving existing correlations. Despite these differences, biological neurons integrate both mechanisms robustly. We aimed to understand the computational mechanisms underlying the successful interaction of neuromodulation and homeostasis (Fig 3). Homeostatic regulation alone can lead to pathological loss of function in response to disturbances. However, when combined effectively with neuromodulation, this loss of function is mitigated. The synergy between neuromodulation and homeostasis enables the robust achievement and maintenance of a desired firing pattern, provided as input to the model.

Additionally, we applied the combined neuromodulation controller and homeostasis to achieve robust gait control in a quadruped robot. Traditionally, gait control and transitions between rhythmic patterns, such as from trot to gallop, have been associated with distinct network architectures, or connectomes. However, such networks face challenges, including susceptibility to disruption, limited adaptability, and difficulty in external manipulation due to their static nature. To overcome these limitations, we developed an innovative approach: integrating neuromodulation to allow dynamic reconfiguration of rhythmic patterns encoded by fixed connectome networks (Fig 4).

By introducing a neuromodulatory network alongside a pre-existing rhythmic network that encodes various patterns in its connectome, we can facilitate seamless transitions between patterns. This transition is achieved by modulating specific neuromodulatory neurons, switching them between bursting and tonic firing states. Remarkably, this pattern shift remains robust, even in the presence of neuronal parameter variability. One significant application of this work lies in the control of locomotion.

Both the neuromodulatory and rhythmic networks utilize half-center oscillators to maintain symmetry, which prevents pattern disruptions caused by neuromodulatory-rhythmic asymmetries. This approach allows us to dissociate the trot and gallop rhythms hardwired in the connectome while providing external modulation through neuromodulatory mechanisms. The implications of this neuromodulatory network extend beyond locomotion control, with potential applications in neuromorphic engineering, including the control of neuromorphic robots and the modulation of spiking neural networks.

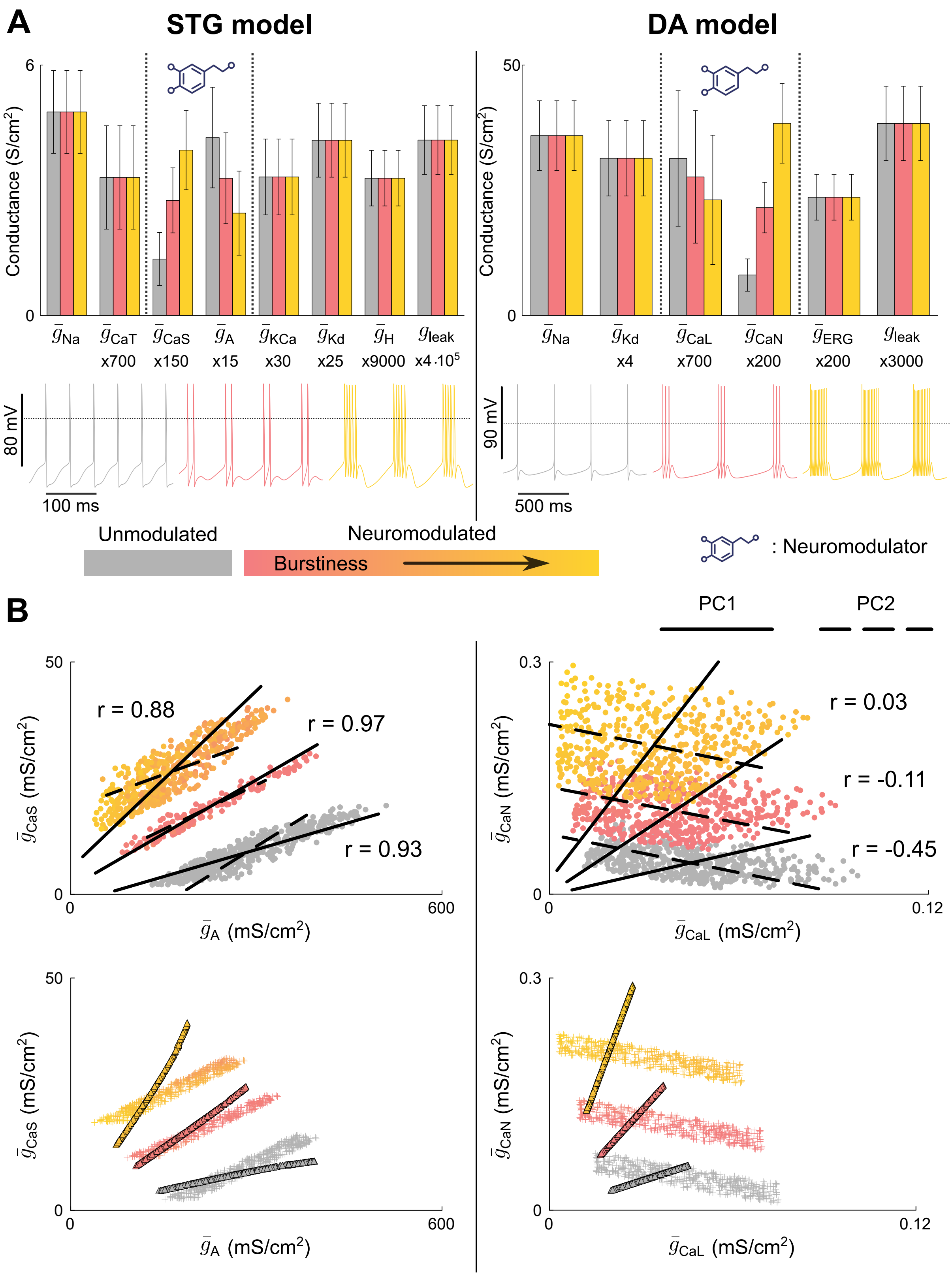

Figure 1: Variability in pairwise correlations in conductance values is neuromodulation-dependent.

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Figure 1: (A) Bar plot of conductance values for custom generated populations in the three phenotypes considered for the STG model (right) and the DA model (left). The dotted line on the voltage traces corresponds to 0 mV.
(B) Scatter plots of full variability custom generated populations in the neuromodulated 2D space for both the STG model (left) and the DA model (right) across three neuromodulated states, along with PC1, PC2, and Pearson correlation coefficients (top). Scatter plots of separated custom generated populations in the neuromodulated 2D space for both the STG model (left) and the DA model (right) across three neuromodulated states (bottom).

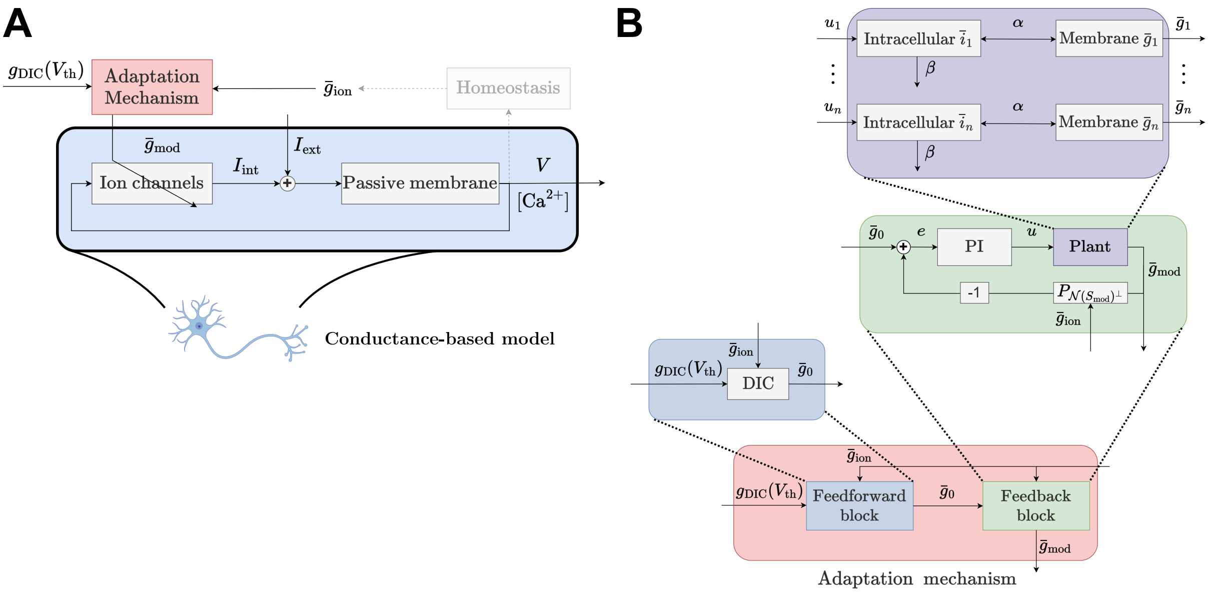

Figure 2: Neuronal excitability and neuromodulation from an adaptive feedback control perspective. A. High level block diagram of the adaptive neuronal controller. The blue block depicts the typical structure of a conductance based model from the feedback control perspective. A neuron is composed of a controller, *i.e.*, voltage and calcium controlled active ion channels, that produces an intrinsic current I_{int}, and a plant, *i.e.*, the passive membrane. *In vitro*, an external current I_{ext} can also be applied to excite the neuron. The red block lumps all the biological mechanisms that regulate ion channel expression and that act as an adaptive layer onto the neuronal controller. Neuromodulator concentration [*nmod*] can be modeled as an input to this adaptive block. **B.** Detailed block diagram of the adjustment mechanism, red block of **A**. It is composed of two sub-blocks: a reference generator (feedforward) block in blue and a reference tracking (feedback) block in green. The feedforward block models a cell signaling cascade that maps target neuronal gains $g_{\text{DIC}}(V_{\text{th}})$ at threshold voltage V_{th} to a reference signal $\bar{g}_0$ for modulated ion channel expression. The feedback block regulates ion channel expression through a classical PI negative feedback control loop of a molecular plant describing ion channel protein translation, trafficking, and membrane turnover.

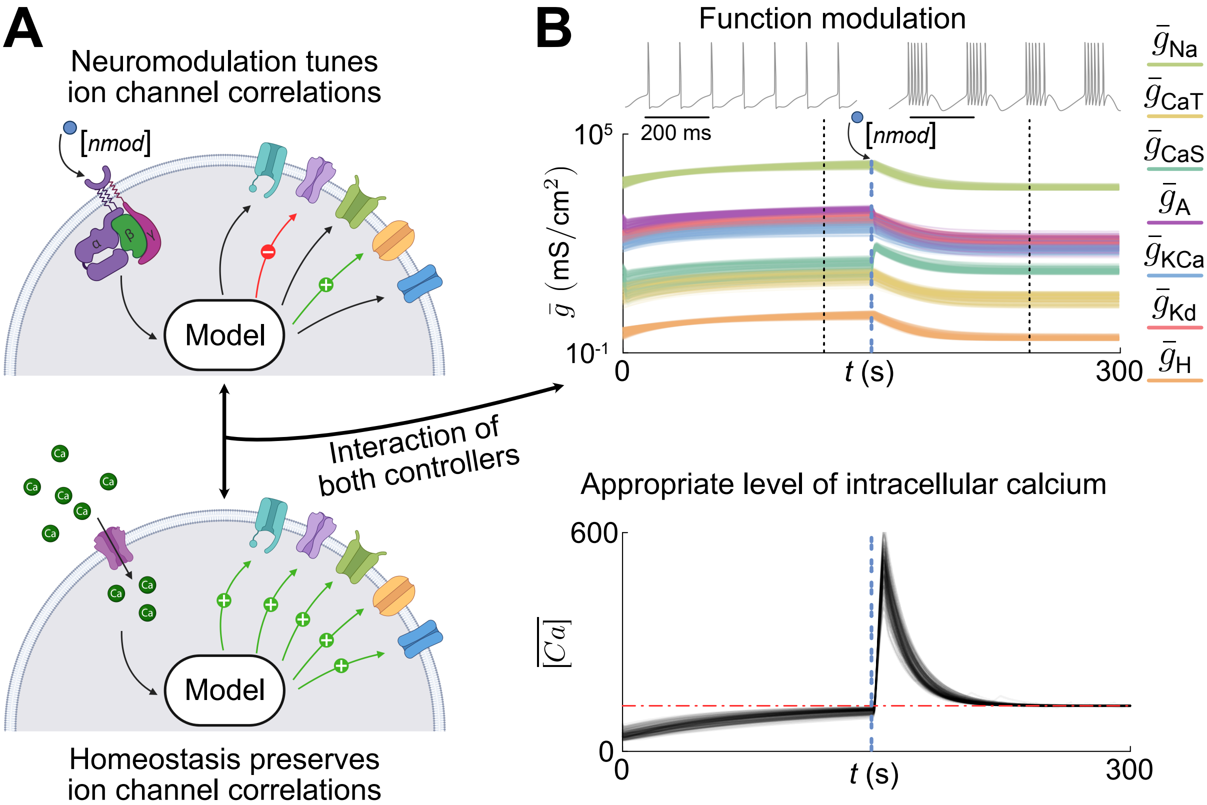

Figure 3: Neuromodulation and homeostasis: complementary mechanisms for robust neural function. A. Schematic of the action of both neuromodulation and homeostasis on ion channels. The former tends to only modify a subset of ion channel, and probably in opposite ways. The latter affect all ion channels in the same direction, hence preserving ion channel correlations. **B.** Simulation results on a 200 STG neurons neuromodulate and homeostatically compensated simultaneously. When neuromodulation occur, ion channel correlation involving $\bar{g}_{\mathrm{CaS}}$ and $\bar{g}_{\mathrm{A}}$ are modified, and the calcium level rises. However, homeostatic compensation does not alter these new correlations and bring the level of intracellular calcium back to baseline.

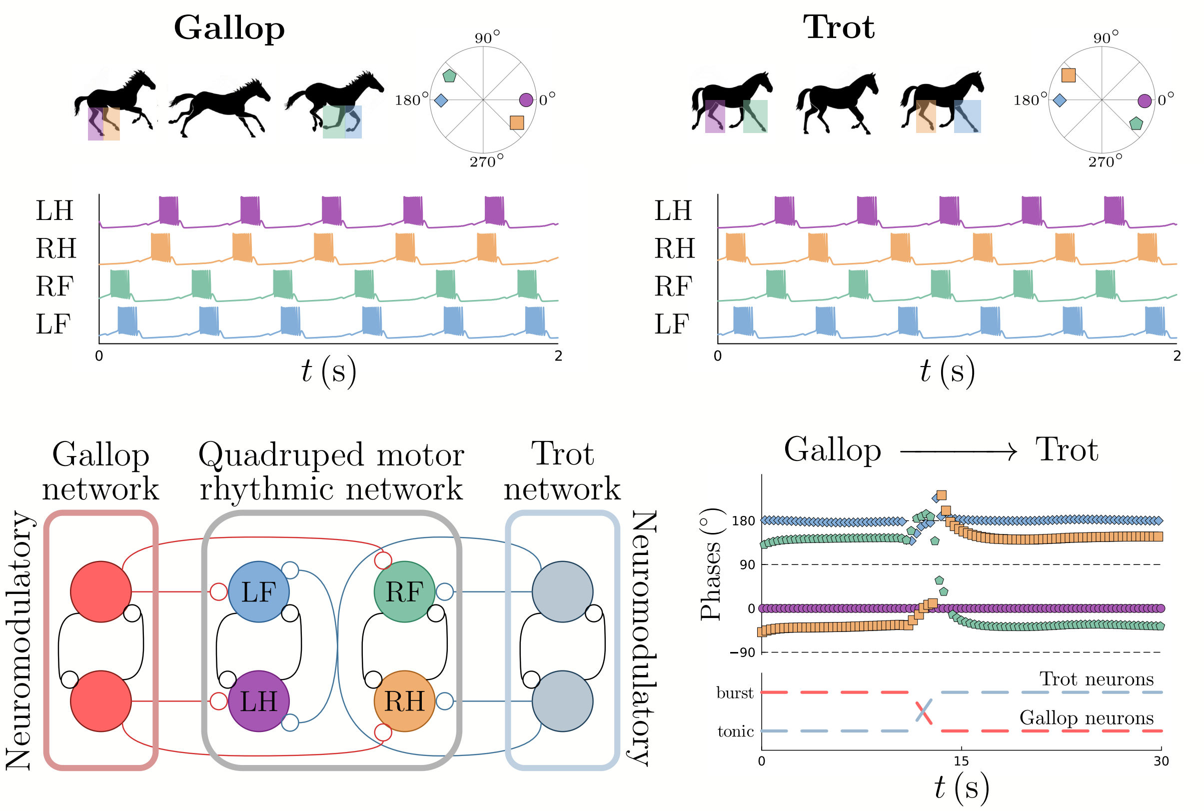

Figure 4: Quadruped gait control using neuromodulation. Typical gait pattern of a quadruped characterized by distinctive phase patterns, along with simulated phase patterns and motor neuron traces (top). Architecture of the fixed connectome neuromodulated network for a robust transition from gallop to trot (bottom left). Simulated dynamic neuromodulation of the gait pattern by modulating the neuromodulatory network (bottom right). LH for left hind, LF for left front, RH for right hind, and RF for right front.