A computational model of altered neuronal activity in altered gravity

Camille Gontier[1*,2], Laura Kalinski[3], Johannes Striebel[4], Maximilian Sturm[3], Zoe Meerholz[5], Sarah Schunk[3,5], Yannick Lichterfeld[3], and Christian Liemersdorf[3]

[1]LIDE Space, Louvain-la-Neuve, Belgium
[2]University of Pittsburgh, Pittsburgh, USA
[3]Department of Applied Aerospace Biology, Institute of Aerospace Medicine, German Aerospace Center, Cologne, Germany
[4]Department of Ophthalmology, Medical Faculty, University of Bonn, Bonn, Germany
[5]University of Bonn, Bonn, Germany

Corresponding author: camille@lide.space

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Data availability statement: Analysis code is available from the following repository: `https://github.com/camillegontier/neuron_microgravity`

Abstract

Electrophysiological experiments have shown that neuronal activity changes upon exposure to altered gravity. More specifically, the firing rate increases during microgravity and decreases during centrifugal-induced hypergravity. However, the mechanism by which altered gravity impacts neuronal activity is still unknown. Different explanations have been proposed: a first hypothesis states that microgravity increases the fluidity of the cell membrane and modifies the properties of the neurons' ion channels. Another hypothesis suggests the role of mechano-gated (MG) ion channels depolarizing the cells during microgravity exposure. Although intuitive, these models have not been backed by quantitative analyses nor simulations. Here, we developed computational models of the impact of altered gravity, both on single cell activity and on population dynamics. Firstly, in line with previous electrophysiological experiments, we suggest that microgravity could be modelled as an increase of the voltage-dependent channel transition rates, which are assumed to be the result of higher membrane fluidity and can be readily implemented into the Hodgkin-Huxley model. Using in-silico simulations of single neurons, we show that this model of the influence of gravity on neuronal activity allows to reproduce the increased firing and burst rates observed in microgravity. Secondly, we explore the role of MG ion channels on population activity. We show that recordings can be fitted by a network of connected excitatory neurons, whose activity is balanced by firing rate adaptation. Adding a small depolarizing current to account for the activation of mechano-gated channels also reproduces the observed increased firing and burst rates. Overall, our results fill an important gap in the literature, by providing a computational link between altered gravity and neuronal activity.

1 Introduction

During spaceflight, humans experience a variety of physiological changes due to deviations from familiar Earth conditions. Specifically, the lack of gravity is responsible for a variety of effects observed in astronauts, including a loss of muscle mass and bone density as a result of musculoskeletal deconditioning [1–3] and modifications of the vestibular and cardiovascular systems immediately upon microgravity exposure [4]. These impairments also include structural as well as functional changes of the brain: an elevated intracranial pressure possibly leading to neuro-ophthalmic anatomical changes (spaceflight-associated neuro-ocular syndrome, or SANS) [5–7], an increased total ventricular volume [7, 8], and a decline in cognitive performance [9–11].

Different experiments have been conducted in altered gravity, in order to further characterize the effect of microgravity on the human body, to assess its deleterious effect on health, and to test mitigation strategies. At the level of organ physiology, they have been used to study, e.g., the effect of spaceflight on brain connectivity [12, 13] and volumes [14, 15]. But experiments in real microgravity are scarce and extremely expensive. Thus, approaches were designed to simulate the effect of microgravity on Earth in ground-based facilities (GBFs). Simulated microgravity on Earth can be accomplished by clinorotation, a constant fast rotation that counterbalances the influence of gravity and exposes the samples to always changing direction of the Earth´s gravity vector. Experiments utilizing ground-based facilities already reported several aspects of neuronal cell behavior. At the

cellular level, experiments using 2D clinostats and random positioning machines (RPMs, also called 3D clinostats) have been used to study the effect of simulated microgravity on several structural features and viability of neuronal cell lines [16]. Simulated microgravity has been shown to enhance the differentiation of mesenchymal stem cells into neurons [17], to enhance and sustain proliferation of oligodendrocyte progenitor cells, contributing to their process extension and migration [18] and also to enhance mitochondrial function in oligodendrocytes [19]. Space conditions also feature increased levels of radiation, which impact astrocyte survival and reactivity [20]. Also, clinostat simulated microgravity experiments facilitated cell migration and neuroprotection after bone marrow stromal cell transplantation in spinal cord injury [21]. Interestingly, microgravity simulated in a random positioning machine enhanced cellular survival rate by maintaining an undifferentiated state of mouse bone marrow stromal cells, which in turn enhanced their potential to be implemented in future neural grafting therapeutic approaches [22]. Additionally, phenotypic markers of neuron/glia culture models have been influenced by microgravity [23]. Since microgravity influences cells by eliminating the gravitational load that they perceive, hypergravity, i.e. increased gravitational load by centrifugal forces, could be utilized to identify gravity-sensitive parameters as well as act as a potential countermeasure against gravity-induced dysfunctions. Hypergravity has been shown to be able to decrease several traits of astrocyte reactivity, an important feature following brain injuries [24]. Taken together, gravity seems to have differential impacts on different types of neuronal cells and needs to be investigated further to yield a comprehensive overview over the effects induced in human spaceflight.

Given the raising interest and need of conducting experiments in microgravity conditions [25] and the cost and complexity of accessing the International Space Station (ISS), different gravity research platforms have been developed on Earth to study the impact of gravity. Different types of 2D clinostats are mostly used to simulate microgravity in the lab [26, 27]. Real microgravity can be achieved by larger gravity research platforms, including drop towers [28], sounding rockets [29, 30], and parabolic flights with large aircraft [31–33], single-engine aircraft [34], and gliders [35]. Similarly, the effect of hypergravity can be studied using centrifuges [24, 36] or an aircraft performing high-bank turns [37, 38]. Alternative space analog studies for human subjects have been employed for decades such as bed rest [39–41] and dry immersion [42, 43], and have also been used to study the effect of altered gravity on gene expression [44] and corticospinal excitability [45]. These studies feature traits such as physiological deconditioning but can not simulate the differential influence of gravity.

Several of these platforms have already been employed to study how neuronal activity is impacted under altered gravity on a human physiological level. Studies performed during parabolic flights have reported a modification of the EEG signal, but not of the participants' cognitive performances, under different micro- and hypergravity conditions [32, 38]. Previous electrophysiological experiments using invasive patch clamp techniques and calcium indicators have revealed different effects, including a higher firing rate and a slower action potential propagation [46], as well as a higher intracellular calcium concentration [27], following exposure to microgravity. Similarly, experiments using Multi-Electrode Arrays (MEA) reported an increase in the firing rates of neurons during microgravity phases and opposing effects upon exposure to hypergravity [36]. These initial changes in spontaneous

action potential spiking frequency were compensated within a minute time range, hinting to an adaptation mechanism.

Different hypotheses have been proposed to explain the increased firing rate during microgravity and the decreased firing rate during hypergravity. First, a possible explanation is that microgravity increases the membrane fluidity of neuronal cells, which modifies properties of the ion channels. More specifically, a previously proposed model [47] hypothesizes that an increase in membrane fluidity modifies the open-state probabilities of ion channels, hence leading to a depolarization of the cell and to an increase in the spontaneous firing rate. Although intuitive, this model is purely qualitative and has not been backed by analytical computations nor simulations. In computational models, a neuron is often described by set of parameters, e.g., their membrane time constant τ_m or their ion channel conductance g. A proper description of how these parameters might be impacted by altered gravity conditions is currently missing.

A second explanation to the increased firing and burst rates in microgravity is based on the role of mechanosensitive ion channels, which operate as gravireceptors in eukaryotic cells [48]. They have been shown to be widely present in the nervous system [49]. These mechano-gated (MG) ion channels have been observed to generate inward currents and to elicit spiking activity in neuronal cells [50]. The modified neuronal activity during periods of altered gravity has thus been attributed to the activity of MG ion channels, whose stretching during acceleration and deceleration phases might impact neuronal activity [36]. But the final effect of an inward current on firing rates is going to strongly depend on the properties of the studied neuron population. Specifically, these MG channels would have different effects depending on whether the observed neurons are isolated or show signs of synchronized activity; whether their recurrent connections are mostly excitatory or inhibitory; or whether they are impacted by non-linear dynamical effects, such as short-term depression or firing rate adaptation. To date, these features have not been characterized in the neuron cell types used in altered gravity experiments.

Our overall contribution is two-fold. First, in section 2.1, we propose that the effect of altered gravity on neuronal dynamics could be represented as an acceleration of the rate at which voltage-dependent ion channels switch from open to close configurations, i.e., as a decrease of the time constants $\tau_n(V)$, $\tau_m(V)$, and $\tau_h(V)$. This is in line with a set of previous observations reporting a modification of the fluidity of the cell membrane in altered gravity, and a direct link between membrane fluidity and ion channels time constants. We verify that decreasing these time constants during in-silico simulations allows to reproduce the increased firing and burst rates observed during altered gravity experiments (section 2.2).

Second, in section 2.3, we propose a computational model to explain the activity of networks of neurons in microgravity. Human stem cell-derived neuronal cells which were already used in microgravity experiments have two remarkable features: they are mostly excitatory, and show slow oscillations of coordinated activity [51]. We show that these features can be accounted for by a network of connected excitatory leaky integrate-and-fire (LIF) neurons which recurrent connections are balanced by firing rate adaptation. We verify that injecting an external depolarizing current into the network (to model the activation of MG channels) also allows to reproduce the increased firing and burst rates observed during altered gravity experiments (section 2.4).

2 Results

2.1 A proposed model of the effect of gravity on ion channels' gating rates

A classically used model of the time evolution of the membrane potential of a neuron, and especially of how action potentials are generated, is called the Hodgkin-Huxley model [52, 53]. It consists of a set of differential equations, describing the evolution of the cell voltage as a function of its ion channels conductances; the evolution of these conductances as a function of the open probabilities of the channels; and the evolution of these probabilities as a function of their voltage-dependent gating rates.

The standard equation describing the time evolution of the voltage V of a single-compartment model of a neuron (see [53] for a detailed derivation) is

$$C_m \frac{dV}{dt} = -i_m \tag{1}$$

where C_m is the specific membrane capacitance (in $F \cdot m^{-2}$) and i_m is the total membrane current. The latter can be expressed as the sum of a delayed-rectified potassium current, a transient sodium current, and a leakage current:

$$i_m = \bar{g}_K n^4 (V - E_K) + \bar{g}_{Na} m^3 h (V - E_{Na}) + \bar{g}_L (V - E_L) \tag{2}$$

where E_L, E_{Na}, and E_K are the reversal potentials, and $\bar{g}_L$, $\bar{g}_{Na}$, and $\bar{g}_K$ are the maximal surface conductances (in $S \cdot m^{-2}$). n corresponds to the probability that a subunit in a potassium channel is activated and hence contributes to the opening of the K^+ channel. Similarly, m and h express respectively the activation and inactivation probabilities of the Na^+ conductances. The time evolution of these gating variables can also be described by a set of differential equations:

$$\frac{dn}{dt} = \alpha_n(V)(1 - n) - \beta_n(V) n \tag{3}$$

$$\frac{dm}{dt} = \alpha_m(V)(1 - m) - \beta_m(V) m \tag{4}$$

$$\frac{dh}{dt} = \alpha_h(V)(1 - h) - \beta_h(V) h \tag{5}$$

where $\alpha_x(V)$ and $\beta_x(V)$ are the voltage-dependent opening and closing rates of the subunit gates, which are usually obtained by fitting on experimental observations (see [53] for a detailed discussion). Eq. 3 can also be written as

$$\tau_n(V) \frac{dn}{dt} = n_\infty(V) - n \tag{6}$$

with

$$\tau_n(V) = \frac{1}{\alpha_n(V) + \beta_n(V)} \tag{7}$$

and

$$n_\infty = \frac{\alpha_n(V)}{\alpha_n(V) + \beta_n(V)} \tag{8}$$

(similar equalities can be derived for the other gating variables m and h). The time constants $\tau_n(V)$ (Eq. 6), $\tau_m(V)$, and $\tau_h(V)$ thus govern the dynamics of channel subunits, i.e., the rate at which they open and close. Interestingly, these time constants have been shown to depend on the structure of the membrane's lipid matrix, and more specifically on the fluidity of the cell membrane [54–56]. In experiments involving recordings from GH3 cells at different solution temperatures, a decrease of the sodium activation and inactivation time constants $\tau_m(V)$ and $\tau_h(V)$ was observed when the temperature increases (Fig. 1 A). These effects were attributed to membrane structural changes, and especially to an increase of membrane fluidity [54]. This link between increased membrane fluidity and decreased gating rates is in line with previous observations made with acetylcholine channel open time [56] and Na^+ channels in giant squid axons [55].

Since several observations have reported an increase in cell membrane fluidity upon microgravity exposure [47, 57], we propose to represent the effect of microgravity on computational models of neurons by a unitless multiplicative constant λ which is applied to the opening and closing rates $\alpha_x(V)$ and $\beta_x(V)$, and hence to the time constants $\tau_n(V)$, $\tau_m(V)$, and $\tau_h(V)$ (Fig. 1 B). The classical gating equations Eqs. 3, 4, and 5 thus become

$$\frac{dn}{dt} = \lambda\left(\alpha_n(V)(1-n) - \beta_n(V)n\right) \tag{9}$$

$$\frac{dm}{dt} = \lambda\left(\alpha_m(V)(1-m) - \beta_m(V)m\right) \tag{10}$$

$$\frac{dh}{dt} = \lambda\left(\alpha_h(V)(1-h) - \beta_h(V)h\right) \tag{11}$$

with $\lambda = 1$ in $1g$, $\lambda < 1$ in hypergravity, and $\lambda > 1$ in microgravity. Fig. 1 B shows that, for a given voltage, increasing the value of λ decreases the gating time constants (left panel) without modifying the gating steady-state levels (right panel). Our approach allows to reconcile two independent observations: the fact that microgravity impacts the cell membrane fluidity [47, 57, 58]; and the fact that membrane fluidity impacts the channels gating time constants [54–56].

2.2 In-silico simulations of single cell activity under altered gravity

Next, we sought to assess the effect of λ on simulated neuronal activity, and especially to verify that a higher value of λ (to represent the effect of microgravity) would reproduce the different features observed during experiments, namely a higher firing rate and burst rate - and that a value of λ set below 1 would reproduce the reduced activity observed following the onset of hypergravity in centrifuge experiments. Simulations were performed using the Brian neuron simulator [59] and the numerical values described in Material and methods.

Fig. 2 A shows the firing rate of a neuron simulated by the Hodgkin-Huxley model as a function of the input current I added to Eq. 2 and of the value of λ in Eqs. 9, 10, and 11. For a given input

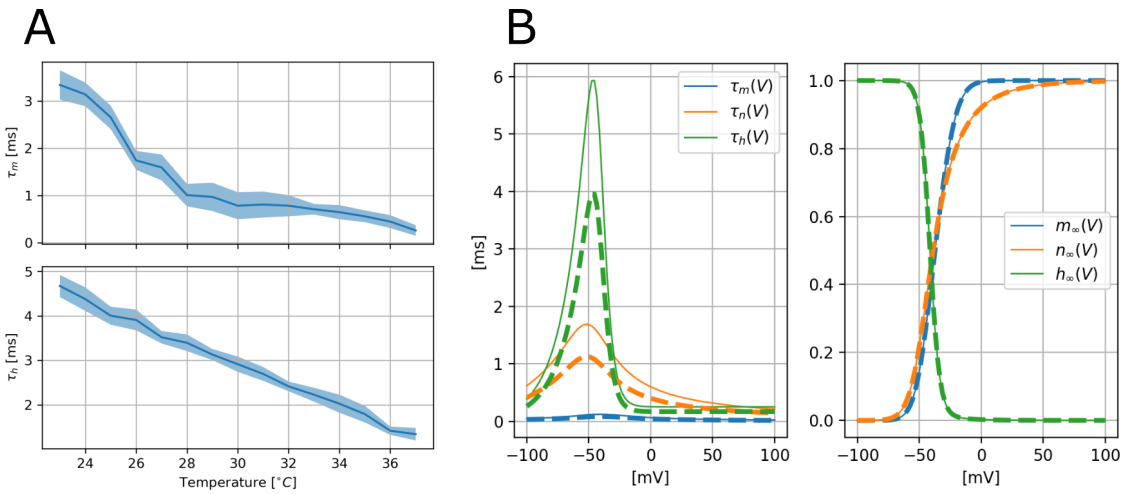

Figure 1: **A proposed model of the effect of gravity on ion channels' gating rates.** A: values of $\tau_m(V)$ (top) and $\tau_h(V)$ (bottom) in GH3 cells at a membrane voltage $V = -20mV$ as a function of temperature. Redrawn from [54]. B: effect of λ on the gating time constants (left) and steady-state levels (right) in the Hodgkin-Huxley model. Solid line: $\lambda = 1$. Dashed line: $\lambda = 1.5$.

current, the firing rate increases with λ, which is in line with experimental observations. We verified that this result is robust to the values used in the simulation by randomly varying the values of the neuron's parameters and checking that a higher value for λ leads to a higher firing rate (Fig. 5). Fig. 2 B shows the time evolution of the voltage of the same neuron for an input current $I = 0.35nA$. A shorter time constant for the gating variables leads to a faster voltage increase, an earlier time to first spike, and a shorter inter-spike interval.

Fig. 2 C shows the effect of λ on the open probabilities of the potassium and sodium channels, i.e., $P_K = n^4$ (left column) and $P_{Na} = m^3h$ (right column). The upper row shows their time evolution in the same condition as in Fig. 2 B, while the bottom row shows the average value of P_K and P_{Na} during 2 seconds of simulation (normalized by the number of action potentials). The end results in the lower row is independent of I, since the firing rate is nearly linear with I for sufficiently large values (see Fig. 2 A). These results show that, under our modelling assumptions, an increase in λ does not increase the opening probabilities of the ion channels, but rather accelerates the time at which the Na^+ conductances activate.

Finally, we also verified that varying the value of the parameter λ allows to reproduce experimental observations not only in isolated neurons, but also in more realistic networks of recurrently connected neurons. To do so, we simulated the activity of a population of $N = 4000$ connected Hodgkin-Huxley neurons (whose parameters are described in Table 1), and verified that increasing the value of λ leads to an increase of both the population firing rate (Fig. 2 D, left), and burst rate (Fig. 2 D, right). In line with previous studies [36, 60], we define a burst as a group of consecutive spikes separated by less than $20ms$. These results are akin to what has been previously observed during altered gravity experiments [36].

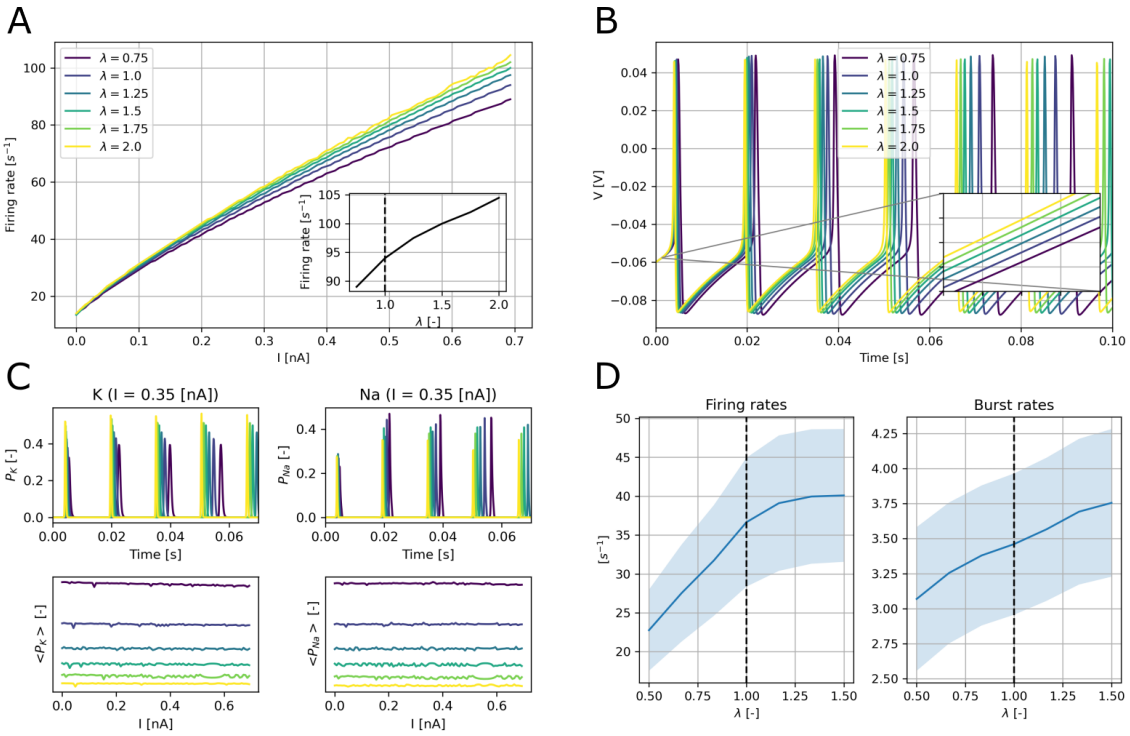

Figure 2: **In-silico simulations of single cell activity under altered gravity**. A: Frequency-current curve of a simulated Hodgkin-Huxley neuron (whose parameters are described in Table 1) for different values of λ. Inset: firing rate as a function of λ for $I = 0.7nA$. B: Time evolution of the membrane voltage for the same cell as in A. C: Evolution of potassium P_K (left column) and sodium P_{Na} (right column) channel opening probabilities for the same cell as in A. Upper row: time evolution of these probabilities. Lower row: Average (over $T = 2s$) value of these probabilities (normalized by the number of spikes during the simulation). D: Mean firing and burst rates (averaged across all neurons) in a network of connected Hodgkin-Huxley neurons as a function of λ. Solid line: mean value over 30 repetitions. Shaded area: s.e.m.

2.3 Observed synchronized bursts can be explained by spike frequency adaptation

In addition to the role of cell membrane fluidity, another hypothesis for the altered neuronal activity involves MG ion channels, whose activation in altered gravity phases would depolarize cells [36]. However, although this explanation makes sense for single isolated neurons, the effect of an inward current on recurrently connected neurons would depend on the properties of the network. More specifically, it would depend on its excitation-to-inhibition ratio, and on the presence of non-linear dynamics, such as short-term plasticity or firing rate adaptation. Such features have not been characterized, and let alone modelled, in the cell lines classically used in altered gravity experiments.

Such cell lines usually include human stem cell-derived neuronal cells (i.e. iNGN cells) (see [51] and [36] for a detailed discussion), which have two main remarkable features. Firstly, their population activity shows synchronized bursts and action potentials (Fig. 3 A, and Fig. 3 C in [51]) at a low frequency (i.e., $< 1Hz$), which is a telltale sign of recurrent connections among neurons. Secondly, the vast majority of these connections are supposed to be excitatory: synchronized activity in iNGN neuronal cell lines is abolished by glutamate receptor blockers, and although inhibitory GABAergic synapses appear in the last stages of cells development, inhibitory neurons only make up approximately 2.3% of the whole population after 30 days of cell growth [61]. This is significantly below the level of inhibition which is classically assumed to be necessary for stable self-sustained population activity [62].

Similar low-frequency oscillations had been previously observed in neuronal populations, especially during sleep, anesthesia, or in-vitro when using a mock cerebrospinal fluid (CSF) [63, 64]. Moreover, different computational models have been proposed to reproduce and explain them. In [63], the authors augment the classical Hodgkin-Huxley model with a slow sodium-dependent potassium current which mediates the transition between up and down states (respectively characterized by higher and lower levels of spontaneous activity). However, this model relies on an important population of inhibitory neurons to stabilize the strong recurrent excitation during the up state. In [65], the authors propose a simpler model (based on LIF neurons) to reproduce these slow-oscillations, but here again the recurrent excitation needs to be balanced by inhibitory neurons. This does not coincide with the very low proportion of inhibitory neurons in the observed population in [36].

However, other phenomena, such as firing rate adaptation [66] and short-term depression [67], have been proposed as ways to stabilize the coordinated activity of connected excitatory neurons without inhibitory feedback. In line with [68], we model the evolution of the voltage potential V in the presence of an injected current I using the adaptive Exponential Integrate-and-Fire model (AdEx), which can be seen as a simplification of Eq. 2 in that it neglects the individual ion-specific dynamics:

$$C\frac{dV}{dt} = -g_L(V - E_L) + g_L\Delta_T \exp\left(\frac{V - V_T}{\Delta_T}\right) + I - w \tag{12}$$

where C is the cell total membrane capacitance, g_L is the total leak conductance, E_L is the effective rest potential, Δ_T is the threshold slope factor, and V_T is the effective threshold potential. The last term w (which is not present in Eq. 2) tends to hyper-polarize the cell following a spike,

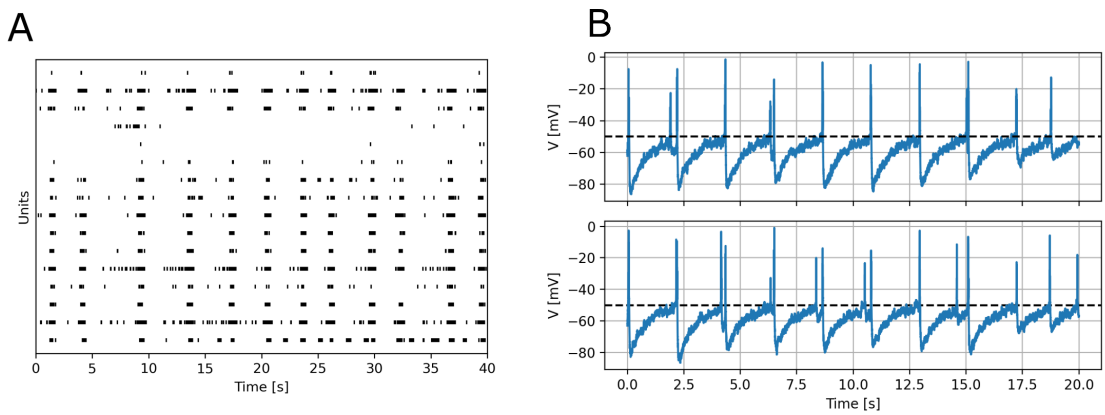

Figure 3: **Observed synchronized bursts can be explained by spike frequency adaptation**. A: Raster plot of detected action potentials on 16 units observed from a human stem cell-derived neuronal network (same setting as in [36]). Spike sorting was performed using SpikeInterface [69] and the MountainSort 5 algorithm [70]. B: Membrane voltage of 2 example cells from a simulated network of connected LIF neurons (without inhibition but with firing rate adaptation). Dashed dark line: action potential threshold. Numerical values used for the simulations are described in Table 2.

hence reducing the probability of a subsequent spike and leading to spike-rate adaptation. It is increased by a quantity b following each spike, and otherwise decays with a time constant τ_w:

$$\tau_w \frac{dw}{dt} = a(V - E_L) - w \tag{13}$$

We verify that the stable self-sustained slow oscillations observed in neuronal cell lines (Fig. 3 A) can be reproduced by a model consisting of a population of LIF neurons recurrently connected by excitatory synapses, whose activity is balanced by firing rate adaptation, without the need for an inhibitory population (Fig. 3 B). Across all simulated neurons, the average firing rate was $5.44 \pm 0.84Hz$ and the average burst rate was $0.66 \pm 0.09Hz$, which is in agreement with the experimental values reported in Figure 2 D and E in [36].

2.4 The model reproduces features of network activity observed in altered gravity

Finally, after having identified the main features of the neuron networks used in altered gravity experiments (i.e., excitatory recurrent connections and population activity stabilized by firing rate adaptation), we verified that injecting an additional current onto this network (to represent the activity of MG channels) allows to reproduce the increased firing and burst rates observed during the dynamic phases of altered gravity experiments. This acceleration-induced current is represented by an extra term I_λ in the right-hand side of Eq. 12:

$$C\frac{dV}{dt} = -g_L(V - E_L) + g_L\Delta_T \exp\left(\frac{V - V_T}{\Delta_T}\right) + I - w + I_\lambda \tag{14}$$

Fig. 4 A shows the raster plot for the same network as in Fig. 3 B, for $I_\lambda = 0pA$ (top) and

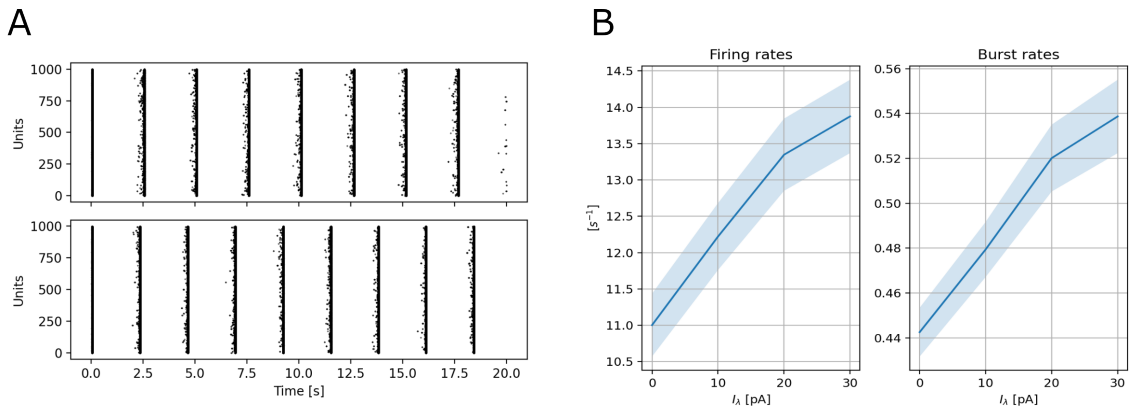

Figure 4: **The model reproduces features of network activity observed in altered gravity**. A: Simulated raster plot of the same network as in Fig. 3 B for different values of I_λ in Eq. 14. Top: $I_\lambda = 0pA$. Bottom: $I_\lambda = 10pA$. B: Firing rate (left) and bursting rate (right) in the same network for different values of I_λ. Solid line: Value averaged over all neurons and over 10 repetitions with random initial cell membranes. Shaded area: s.e.m.

$I_\lambda = 10pA$ (bottom). Fig. 4 B shows the main firing rate (left) and burst rate (right) of the network (averaged over all neurons) as a function of I_λ. Both the firing and burst rates increase with I_λ, which is in line with experimental observations. More specifically, hypergravity experiments using the same hiPSC-derived neurons on the DLR human centrifuge in Cologne, Germany have reported a decreased neuronal activity compared to baseline recordings during exposure to 6g hypergravity (as detailed in section 2.2), but also an increased activity during both the ramp-up and ramp-down phases of the centrifuge (Fig. 2 D and E in [36]). These ramp-up and ramp-down phases correspond to a dynamic modification of the perceived acceleration, during which MG channels are likely to be activated and to contribute to the depolarization of the cells.

3 Discussion

Experiments in an altered gravity environment are a fundamental part of many branches of applied sciences, including biology, physiology, and space medicine. Astronauts that were evaluated after return to Earth showed markedly changed brain physiology that poses risks for human performance and thus safety in spaceflight scenarios [13, 15, 71]. Identifying the underlying mechanisms and pathways that are responsible for this complex phenotype of cognitive impairment induced by spaceflight is necessary to ensure safety and optimal performance on future human space missions.

Several electrophysiology experiments utilizing gravity research platforms for real microgravity (e.g., using drop towers or sounding rockets) have reported an increase of the firing and bursting rates of neuronal cells following the onset of microgravity. A seminal explanatory model states that exposure to altered gravity modifies the physical properties of the cells' membrane, ultimately altering their dynamics. Previous theoretical studies had already shown how, for the same stationary input current, firing rate can be modulated by the dynamical properties of the cell membrane to

perform prospective decoding [72]. Moreover, in experiments involving populations of neuronal cells exposed to centrifuge-induced hypergravity, lower firing and burst rates were observed during the 6g hypergravity phase compared to baseline; but an opposite effect was observed during the ramp up (i.e., the transition from 1g to hypergravity) and ramp down (i.e., the transition from hypergravity to 1g) phases. This effect was attributed to MG ion channels, whose stretching during acceleration and deceleration phases might impact neuronal activity [36].

Although intuitive, these hypotheses (i.e., the modification of the properties of the membrane, and an excitatory current induced by MG channels) may not hold in the presence of non-linear dynamics and recurrent cell connections, and have not been validated by computational nor numerical analyses. Here, we fill an important gap in the literature, by developing models of the effect of altered gravity on neuronal dynamics that encompass and confirm previous hypotheses. In line with previous studies on cell membrane properties, we model the effect of microgravity as a modification of the channel subunits time constants (Fig. 1) and verify that it allows to reproduce altered firing and bursting rates, both in single neurons (Fig. 2 A to C) and in connected networks (Fig. 2 D). Regarding the influence of MG channels, the effect of an additional excitatory current is easy to analyse for single cells, but needs to be assessed in networks of connected neurons. We show that the main features of the cell lines used in altered gravity experiments (i.e., synchronized activity with low-frequency bursts) can be reproduced by a network of connected excitatory cells stabilized by firing rate adaptation (Fig. 3), and that its firing and bursting rates will increase upon addition of an additional excitatory current (Fig. 4).

Both hypotheses (i.e., modification of the cell membrane fluidity, and activation of MG channels) are complementary in explaining the altered neuronal activity observed during altered gravity experiments. The increased activity during static short-tem microgravity and the decreased activity during static hypergravity are coherent with an increase (resp. a decrease) of membrane fluidity. This is well reproduced by our model, where firing and bursting rates are increased compared to baseline for a higher influence of microgravity $\lambda > 1$ (i.e., when the time constant for channel dynamics is accelerated) and decreased for $\lambda < 1$ (Fig. 2). In addition, the increased activity observed during dynamic alteration of gravity (i.e., both during the ramp-up and ramp-down phases of centrifuge experiments) is coherent with a depolarizing activation of MG channels, which we model as an additional excitatory intracellular current (Fig. 4). Future experiments could be designed to further isolate both effects, e.g., by silencing MG channels or modifying the experimental medium to act on membrane fluidity. Patch clamp and sequencing experiments could help in further specifying the type and dynamics of the MG channels present.

The models we use in our simulations (and especially the Hodgkin-Huxley model) rely on large number of free parameters (see Tables 1 and 2). However, these parameters can be easily constrained to small ranges of biologically plausible values. Seminal papers on conductance-based neurons [73] and firing rate adaptation [68] have proposed biologically plausible values for these parameters, which we based our simulations on. Some of these parameters (namely the adaptation parameters τ_w and b, and the connectivity parameters I, p, and w_e in Table 2) were adjusted to match the specificities of the studied neurons (and especially to reproduce the synchronized, low-frequency bursting activity observed in Fig. 3 A and in [36]). We verified that these values are in line with these previously

used in seminal studies on modeling neuron networks [62]. In Tables 1 and 2, the values for the capacitance C_m and leakage reversal potential E_L are in line with these reported for actual iNGN neuronal cells [74]. Future experiments could focus on estimating the exact value of the other parameters (i.e., the ion-specific parameters in Table 1, or the connectivity parameters in Table 2) for iNGN neurons using dedicated intracellular recordings. Overall, we argue that our results are based on realistic values of the simulation parameters, and that they are robust to reasonable variations in these values.

The altered response observed in cell cultures usually immediately follows the onset of microgravity. However, in longer altered gravity experiments (i.e., involving sounding rockets or centrifuges), the initial raise in firing rate re-adapts after about 40 seconds and returns to baseline levels. The computational models outlined here only account for the former (i.e., the short-term modification of neuronal activity following exposure to microgravity, for which quantitative analyses and numerical verification were lacking). To account for the later (i.e., the relaxation of neuronal activity to baseline levels following prolonged exposure to altered gravity), many different phenomena and models have been proposed to explain response to activity perturbation, including individual neurons spike frequency adaptation [75–77] and synaptic homeostatic plasticity [78–80].

The current studies clearly show the gravity-dependent response of spontaneous activity of neuronal networks of human iPSC-derived neurons (iNGNs), endorsing the importance of further investigations of neuronal activity and its adaptive responses to micro- and hypergravity including acceleration and deceleration phases. Therefore, it would be interesting to design microgravity experiments (e.g., involving parabolic flights) to investigate the effect of repeatedly varying g-loads on the electrophysiological activity of cultured neuronal networks. It will be of particular interest to observe whether the cells respond to the alternating hyper- and microgravity stimuli continuously, or if adaptation processes occur.

The objective of studying cells in altered gravity is two-fold. First, it allows to understand and alleviate symptoms that future astronauts may encounter during spaceflights. Secondly, it may also pave the way towards more efficient treatments for patients on Earth: for instance, rats bone marrow stromal cells cultured in simulated microgravity have been shown to be more efficient for cell-based therapies than cells cultured under Earth's gravity [21]. Functional electrophysiological output is a central part of neuronal health and its dysregulation is a hallmark of neurodegenerative or neurological disorders [81, 82]. Considering that altered neuronal transmission may also lead to diminished human performance in space, it is of high importance to understand the influence of altered gravity at the cellular and network level to increase safety and maintain human performance especially during long-term space exploration missions.

4 Material and methods

Name	Value	Description
A	$2 \cdot 10^{-4} \cdot cm^2$	Membrane area
C_m	$1\mu F \cdot cm^{-2} \cdot A$	Membrane capacitance
$\bar{g}_L$	$5 \cdot 10^{-5} S \cdot cm^{-2} \cdot A$	Membrane conductance
$\bar{g}_K$	$100 \cdot 10^{-3} S \cdot cm^{-2} \cdot A$	Potassium conductance
$\bar{g}_{Na}$	$100 \cdot 10^{-3} S \cdot cm^{-2} \cdot A$	Sodium conductance
E_L	$-60mV$	Leakage reversal potential
E_K	$-90mV$	Potassium reversal potential
E_{Na}	$50mV$	Sodium reversal potential
V_{th}	$-63mV$	Spike threshold potential
τ_e	$5 \cdot 10^{-3} s$	Decay time constant (excitatory synapses)
τ_i	$10 \cdot 10^{-3} s$	Decay time constant (inhibitory synapses)
w_e	$6nS$	Conductance quantum (excitatory synapses)
w_i	$67nS$	Conductance quantum (inhibitory synapses)
E_e	$0mV$	Reversal potential (excitatory synapses)
E_i	$-80mV$	Reversal potential (inhibitory synapses)

Table 1: Parameter values used in Fig. 2 (similar values as in [73]). The detailed equations for the Hodgkin-Huxley model and the COBA synapses used in Fig. 2 are also described in [73].

Name	Value	Description
C	$200pF$	Total capacitance
g_L	$10nS$	Total leak conductance
E_L	$-60mV$	Effective rest potential
Δ_T	$2mV$	Threshold slope factor
V_T	$-50mV$	Effective threshold potential
a	$2nS$	Adaptation conductance
τ_w	$800mV$	Adaptation time constant
b	$30pA$	Spike triggered adaptation
V_r	$-46mV$	Reset potential
I	$102pA$	Injected current
p	0.02	Connection probability
w_e	$0.5mV$	Post-synaptic potential amplitude

Table 2: Parameter values used in Fig. 3 and Fig. 4. Values for $C, g_L, E_L, V_T, \Delta_T, a$, and V_r are the same as these used to simulate regular bursting in [68]. Values for τ_w, b, and I were adjusted to fit the long inter-burst intervals observed in experimental data (Fig. 3 A).

5 Supplementary material

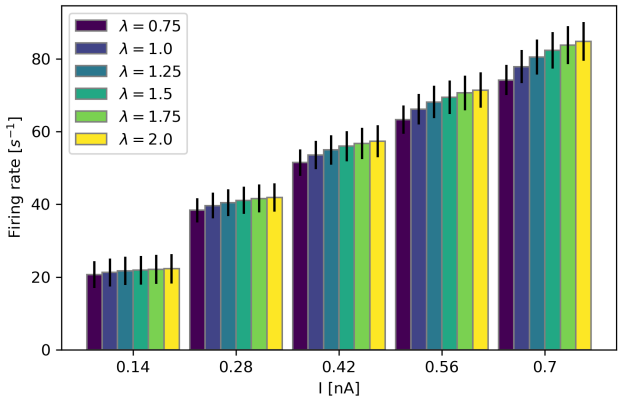

Figure 5: **Robustness analysis.** Same results as in Fig. 2 A, with results averaged over 100 independent repetitions. For each repetition, the value of each simulation parameter was randomly drawn from a uniform distribution with bounds ±10% of the nominal values listed in Table 1. Vertical bars: s.e.m.

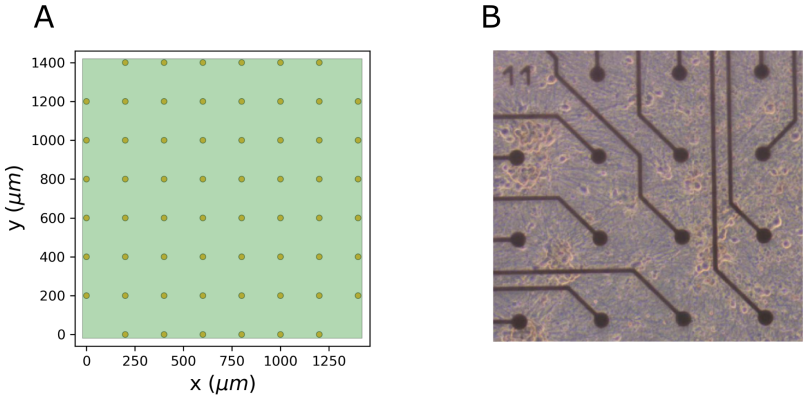

Figure 6: **Layout of the recording electrodes**. A: Population activity was recorded using Multi-Electrode Array (MEA) chips featuring 60 individual electrodes (60MEA200/30iR-ITO chips, MultiChannel Systems, Germany). The 60 electrodes have a diameter of $30\mu m$ and are separated by $200\mu m$. The extracellular voltage recorded by each electrode is sampled at 25 kHz. Each neuronal population was recorded using 2 sets of MEA chips simultaneously. B: Zoomed phase contrast microscopy image of an exemplary MEA chip showing different neurons. Reproduced from [36].

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