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Low-Frequency Tibial Neuromodulation Increases Voiding Activity - a Human Pilot Study and Computational Model

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eLife Assessment

This **important** study investigates frequency-dependent effects of transcutaneous tibial nerve stimulation (TTNS) on bladder function in healthy humans and, through a computational model, shows that low-frequency stimulation accelerates, and high-frequency delays, the urge to void. The integration of experimental and modeling approaches provides a **solid** proof-of concept foundation for clinical trials targeting urinary retention. However, concerns were raised about over-interpretation of modest effects and the limited physiological validity of the computational model, and the need for replication in clinical populations. Some conclusions, particularly in the abstract, could be further tempered to better align with the strength of the available evidence.

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Abstract

Despite widespread clinical adoption for disorders of incontinence such as overactive bladder, there remain unknowns surrounding the mechanism that underpins Tibial Nerve Stimulation (TNS). Current understanding suggests that TNS counteracts incontinence by the inhibition of brainstem and spinal cord activity. How this inhibition alters bladder function is not fully understood. We hypothesize that the supraspinal components of the system act as a high-pass filter, allowing voiding signals to proceed only when bladder filling reaches a critical level. Testing this hypothesis may explain how TNS is able to induce both an inhibitory and a little-explored excitatory effect on bladder activity in response to high-frequency (20 Hz) and low-frequency (1 Hz) stimulation, respectively. We performed a single-blinded trial in healthy human participants administered high and low-frequency Transcutaneous TNS. We also developed a computational model of the lower-urinary tract and control circuit to study the frequency-dependent effects of TNS. For the first time, we report a frequency-dependent effect of TNS via the ability to alter urge perception and up-regulate and down-regulate bladder activity, corroborating model predictions. These results provide a foundation for the development of targeted and effective TNS therapies, benefiting from *in silico* models. We hope that future clinical research will determine the efficacy of low-frequency TNS as a non-invasive treatment option for urinary retention.

Significance Statement

This work describes an experimental study supported by a novel computational model that for the first time captures the frequency-dependent effects of tibial neuromodulation on the urinary control system in humans. The findings of the work provide critically important evidence for the

role of the brainstem as a filter, which may explain the little-explored excitatory effect of tibial neuromodulation on bladder activity. These results have considerable clinical implications in the treatment of urinary retention, a condition for which there are at present very few non-invasive treatment options available.

Introduction

Micturition is regulated by a dynamic reflexive process that switches between periods of storage and release of urine held in the bladder. This cycle is mediated by a complex network of autonomic and somatic neurons in the central and peripheral nervous systems [1–3].

The tibial nerve has a significant influence on micturition [4, 5], because projections from the tibial nerve to the spine inhibit afferent sensory neurons [6–8] and potentially several midbrain nuclei, including the Periaquaductal Grey (PAG) and Pontine Micturition Centre (PMC) [9–11]. This inhibition has been shown to act through both classical and opioidergic mechanisms [12]. This differential mechanism of action likely explains the ability of the tibial nerve to inhibit both the nociceptive and non-nociceptive activity of the bladder [13].

The ability of the tibial nerve to down-regulate bladder activity has led to the formal adoption of tibial nerve stimulation (TNS) as a clinical intervention for the management of symptoms of the lower urinary tract [13, 14]. Non-invasive transcutaneous TNS (known as TTNS) is used as a clinically approved treatment for the management of incontinence and overactive bladder [15]. However, despite the widespread adoption of this technique in clinical practice, key aspects of the dose-related efficacy of TTNS remain unexplored. Specifically, preliminary feline evidence suggests that there may be a frequency-dependent effect of TTNS where low-frequency stimulation is able to up-regulate bladder activity, in contrast to high-frequency stimulation, which downregulates it [16–18].

We aimed to explore whether the preliminary evidence for a presently underexplored frequency dependence manifested as a functional effect in a human population and potentially shed light on a possible mechanistic explanation for the effect. We sought to achieve these goals with a two-pronged approach: conducting a pilot study in a healthy human population and developing a detailed computational model that can explain such frequency dependence *in silico*.

Therefore, we conducted a single-blind randomized control trial in a healthy adult population. In addition, we included a washout period to study if any observed effects linger in nature. With our computational model of the lower urinary tract and its controlling neural circuits, we simulated the bladder control network.

It is hypothesized that by varying only the frequency of applied TTNS, it will be possible to selectively up-or down-regulate bladder behavior in healthy adult humans. Moreover, we hypothesize that modeling the system computationally will allow elucidating the specific mechanism behind the effect.

We found that a frequency-dependent up-or down-regulation of bladder activity could be induced in a healthy human population. Furthermore, our computational findings corroborated these results and indicated that the effect may be mediated by brainstem-specific tibial projections and supported by spinal inhibition.

We propose a mechanistic framework for this effect based on the idea of filtering afferent sensory activity. These results reveal for the first time the presence of a frequency-dependent effect of TTNS in humans and provide a considerable foundation for future clinical research pertaining to the treatment of several disorders of the lower urinary tract.

Methods

Ethical Approval

The study was approved by the local ethics committee of The University of Edinburgh (ID: 977504). Forty-eight people were recruited under three experimental conditions. Before participation, they gave their full informed consent in writing.

Study design

A graphical overview of the study can be seen in Fig. 1. In summary, the study was a single-blind design between subjects. Participants were pseudo-allocated to one of three groups such that the sample size across groups remained equally distributed (see below). Participants were not informed of this decision to ensure blinding was maintained.

To ensure a relatively stable bladder baseline across the sample, several measures were taken. First, participants were asked to restrict their fluid intake for two hours and caffeine or nicotine for 12 hours before the beginning of the experiment - caffeine or nicotine affect urination [19, 20]. Second, participants were asked to empty their bladder as much as they were reasonably able upon arrival, before ingesting 750 ml of water.

Participants underwent a 30 minute “digestion period” during which they were able to undertake any activities they wanted as long as they remained seated. During this period, they were prepped for neurostimulation: Any leg hair was shaved from the ankle and lower leg using a sterile disposable razor, and the stimulation site was disinfected using a 70% isopropyl alcohol solution to remove contaminants.

To ensure that the objective outcome of the study could be recorded in all cases, after the digestion period, participants were told to inform researchers when they first felt the urge to urinate. Electrodes (*Med-Fit 50mmx50mm Hydrogel Electrodes*) were applied to the right leg 1 *cm* posterior to and 10 *cm* superior to the medial malleolus in accordance with established TTNS protocols [13]. Participants received stimulation (*Digitimer DS7A High Voltage Constant Current Stimulator*) until they felt the urge to urinate. The specific parameters of the stimulation differed according to group allocation (see fig. 1):

- Group A: 1 Hz, 200 *μs* pulse width, motor threshold.
- Group B: Control group, without stimulation, though they still underwent skin preparation.
- Group C: 20 Hz, 200 *μs* pulse width, motor threshold.

In all cases, any applied stimulation was monophasic and administered at a standard voltage (max 300V, see supplementary materials) with a current sufficient to induce flexion or fanning of the big-toe. The current was slowly increased until the first sign of this motor activity was observed.

Upon reporting the urge to urinate, the time-elapsed was recorded and the participants were asked to rate how intense they felt their urge according to a validated questionnaire [21] (see Supplementary Materials).

Washout Period

To analyze the presence of lingering effects and to explore whether the hypothesized excitatory effect was reversible, an additional washout period was included in the study methodology, as shown in Fig. 1). After reporting the urge to urinate, participants were asked if they would be willing to take part in an additional optional experiment. If they agreed, participants underwent an optional 10 minute extension to the study where:

- Group A received 20 Hz stimulation, 200 *μs* pulse width, motor threshold.
- Groups B & C were asked to remain seated for 10 minutes and no stimulation was applied (the electrodes were disconnected from the stimulator).

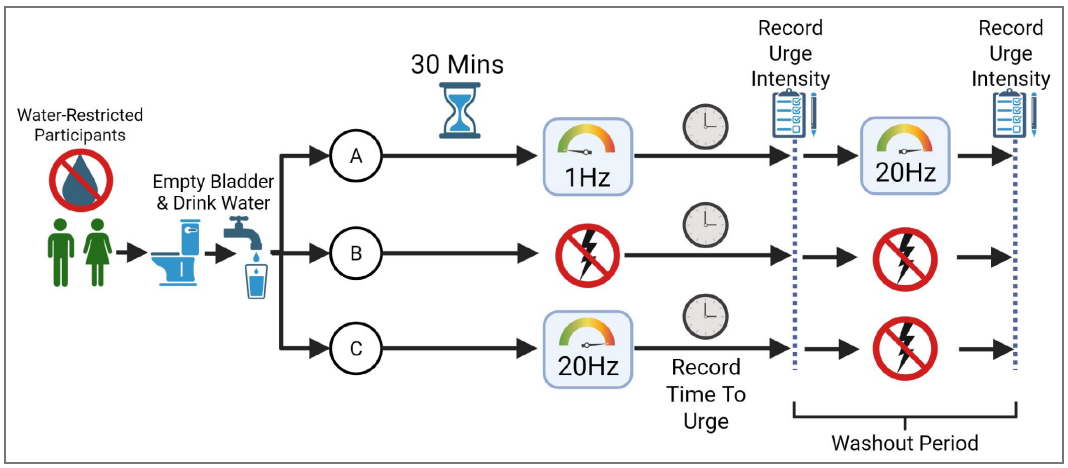


Figure 1. Experimental Study Methodology

Shown is a high-level overview of the study used to validate the frequency-dependent effects of TTNS predicted by the computational model. Participants were healthy adults (18+) asked to abstain from any nicotine/caffeine for 12 hours, and any fluids for 2 hours before the study. Upon arrival they were asked to ingest 750 ml water after emptying their bladder. A 30 min digestion period was employed before stimulation. Participants were pseudo-randomly allocated between groups, and were blind to the condition. Created with *BioRender.com*.

To maintain the blinding of participants to their initial group allocation, participants in groups B and C were told that the optional experiment would simply involve sitting without the electrodes on for ten minutes. At this point the electrodes were removed from the ankle (to ensure they were aware no stimulation was applied) and the washout period initiated for these groups. Individuals in group A were informed that the optional experiment would involve administering a different kind of stimulation which would feel different.

After this washout period, a final urge intensity score was obtained before the end of the study.

Model Topology

The simulated network was composed of a set of interconnected neuronal units each comprising 100 individual neurons modeled in terms of conductance, or Poisson point processes that generated simulated action potentials (see Neuronal Model). The topology of the model was adapted from previous work that simulated reflex voiding [22]. The circuit remained broadly unmodified, with the exception of several key modifications, including simulation of the tibial nerve and its projections (see Fig. 2). We focus on normal bladder function; therefore, the nociceptive bladder afferents were not included in our model. To simulate the effects of opioidergic and classical inhibition, neurons and synapses were represented in terms of ionic conductance. The biophysical model used to represent the bladder was adapted from [23](see Bladder Model).

The tibial nerve was modeled to project to second-order sensory afferents (via classical inhibitory synapses) and all neuronal units within the PAG and PMC (via opioidergic inhibitory synapses) as shown in Fig. 2. To provide fine control over the level of tibial modulation applied to the system, the firing rate of the neuron and its projections were clamped and, if required, could be severed by disconnecting synaptic junctions. This ensured that while in a severed state, the neurons though still modeled had no impact on post-synaptic targets.

Bladder Model

The model used a biophysical representation of the bladder adapted from the work published by Lister et al. [23] modified to allow integration with the conductance-based representation of the micturition control circuit used in the present work. In summary, for each step of the simulation (t), the firing rates of three key efferents: Pelvic, Hypogastric, and Pudendal were used to calculate the internal activation constants - $\omega_{e/i/s}$ - which governed detrusor excitation/inhibition, and sphincter activation respectively. From these values, the bladder state could be calculated and the internal detrusor pressure ($P_B(t)$) used to update the afferent neuronal activity (the main input for the control circuit; see Fig. 2). To ensure computational efficiency, the sampling rate of the model was set at 50 Hz, with each step of the simulation (Δt) representing a 20-ms increment. A detailed description of the model can be found in [23].

Computational Model Fitting

To fit the synaptic weights of the model (see Supplementary Materials), we utilized real bladder pressure and neural data from a previously published work (see Jabbari et al. 2019[24]. In brief, the training data was obtained from intact adult male Wistar rats. The dataset was comprised of bladder pressure and volume information, and extracellular neuronal recordings obtained from between the L6 to S1 spinal cord.

Neuronal Model

The model was built using the Brian2 package for Python [25]. The relationship between the pelvic afferent firing rate and bladder pressure was calculated according to an empirically derived relationship determined previously [26] where the firing rate of the primary bladder afferent at each moment ($F(P_B(t))$) was defined as:

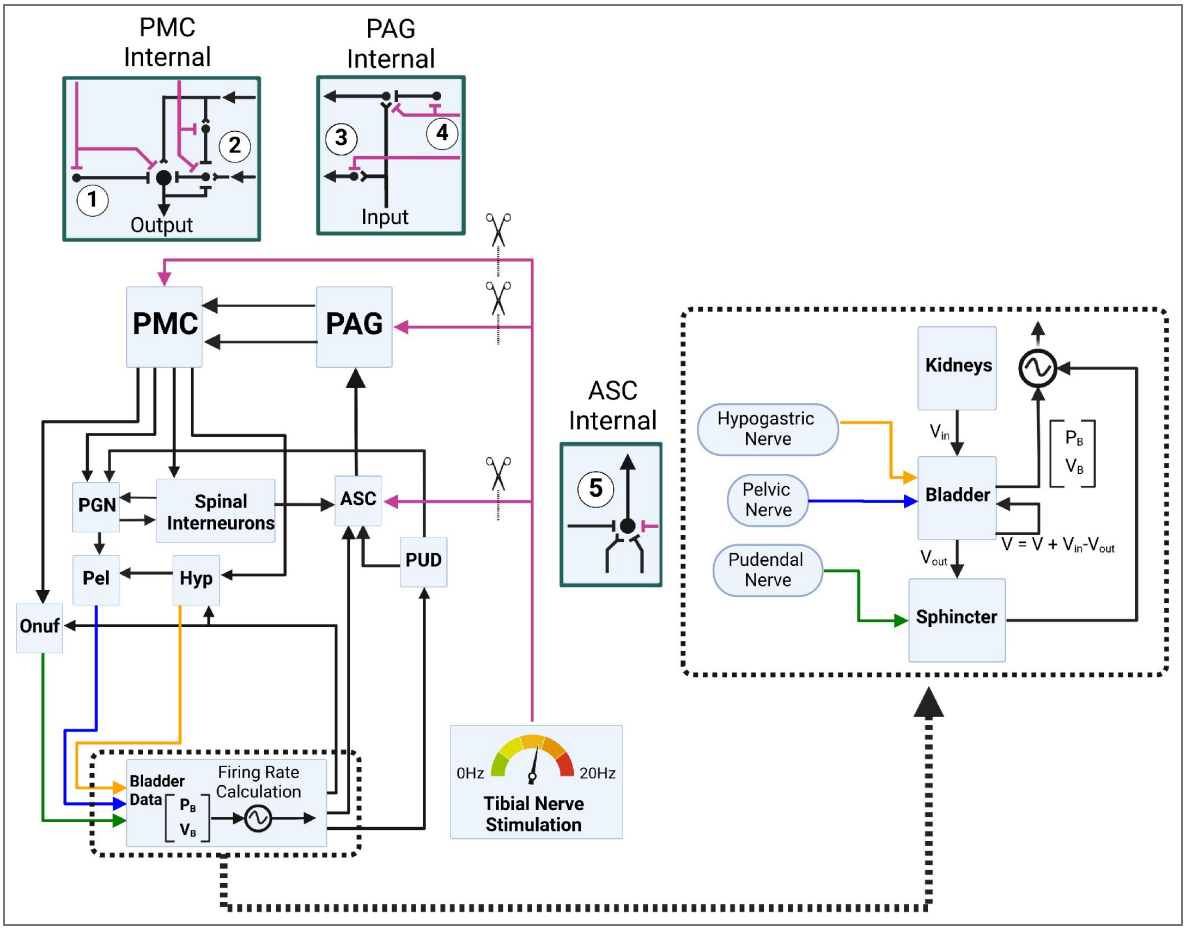


Figure 2. Overview of the Computational Bladder Control Model

A. Shown is a block diagram of the simulated neuronal circuit and bladder model. The model used a modified biophysical representation of the bladder produced previously (dashed box, [23]) which used the firing rates of the pelvic (blue), hypogastric (orange), pudendal (green) efferents to calculate bladder state. Tibial input used to modulate the circuit shown in purple. PMC: Pontine Micturition Centre, PAG: Periaquaductal Grey, PGN: Preganglionic Bladder Neurons, ASC: Ascending Interneurons, PUD: Pudendal Afferent, Onuf: Onuf's Nucleus, Pel: Pelvic Efferent, Hyp: Hypogastric Efferent. Scissors represent possible severed projections. Labels 1 - 5 represent key regions of tibial modulation using opioidergic (1 - 4) or classical (5) inhibitory mechanisms. Created with *BioRender.com*.

$$F\left(P_B(t)\right) = \begin{cases} -3\times10^{-8}P_B(t)^5 + 1\times10^{-5}P_B(t)^4 - 1.5\times10^{-3}P_B(t)^3 \\ +7.9\times10^{-2}P_B(t)^2 - 0.6P_B(t) \\ 0Hz \end{cases} \begin{matrix} F\left(P_B(t)\right) \\ \geq 0Hz \\ F\left(P_B(t)\right) \\ < 0Hz \end{matrix} \quad (1)$$

All neuronal units, with the exception of primary bladder afferents and the tibial nerve, were simulated using a Conductance-Based Adaptive Exponential Integrate-and-Fire (CAdEx) model [27] modified to include opioidergic inhibition. Through the inclusion of an additional input current (I_{ap}) neurons could be made tonically active where required. This was particularly important in the simulation of PAG and PMC, where inhibitory feedback mechanisms reliant on tonic activity were present.

The tibial nerve and primary bladder afferents were simulated as Poisson point processes which generated simulated neuronal spikes at a pre-specified rate. Doing so allowed the firing rate of the pelvic afferent to be set according to the aforementioned mathematical relation and the firing rate of the tibial nerve to be clamped to a specific value (representing external stimulation of the nerve).

Each *unit* within the network was composed of 100 simulated neurons connected via random synapses (where no autapses were permitted) to introduce noise into the system.

Membrane potential (v) was defined as

$$C_m\frac{dv}{dt} = g_L\left(E_L - v\right) + g_L\Delta_t\exp\left(\frac{v - v_{th}}{\Delta_t}\right) + g_A\left(E_A - v\right) + I_{ap} + I_{syn} \quad (2)$$

where C_m was the membrane capacitance; g_L the leakage current & E_L its associated reversal potential; g_A was the adaption current and E_A its reversal potential; I_{ap} the input to tonically firing neurons; and I_{syn} the input from synaptic connections. Also included were terms that govern the action potential (v_{th} and Δ_t). As with the original definition proposed buy Gorski et al,[27], g_A was defined as:

$$\tau_A\frac{dg_A}{dt} = \frac{\overline{g_A}}{1+\exp\left(\frac{v_A - v}{\Delta_A}\right)} - g_A \quad (3)$$

where the rate of change of the current was determined by a rate constant (τ_A), the subthreshold adaption parameters (v_A and Δ_A), and the maximum subthreshold adaption conductance ($\overline{g_A}$). The model also included a post-spike reset mechanism, which too remained unchanged.

$$\text{if } v \geq 0mV \text{ then } \begin{cases} v & \mapsto v_R \\ g_A & \mapsto g_A + \delta g_A. \end{cases} \quad (4)$$

Upon spiking, the membrane potential was reset to its resting state (v_R), and the adaption current increased (δg_A). The purpose of the adaption current was to allow modeling of the spike-frequency adaption. Tonically active neurons did not display spike-frequency adaption to ensure that their activity remained consistent.

Tibial Nerve Stimulation Analysis

To computationally analyze the impact of tibial modulation, simulations were performed in a range of modulatory states. Tibial modulation was applied at 21 different frequencies (0-20 Hz, in 1 Hz increments, $N_{repeats}$ = 10 in each condition) to a simulation of 500-second duration (where each 500 second simulation was run separately). Any aspect of simulated bladder function or circuit activity could be recorded during these simulations. Where neuronal firing rate activity was analyzed, instantaneous firing rates for each recorded time step were smoothed (1st-order

Butterworth filter, with a 1 Hz cutoff). To analyze the effect of specific tibial projections, projections to regions of interest could be severed as necessary to prevent the formation of synaptic connections.

Results

Participants Display a Frequency-Dependent Sensory Response to TTNS

Before statistical analysis was conducted, several datapoints had to be removed from the dataset due to:

- A failure to abstain from caffeine/nicotine before participation (n = 1, Group A)
- Presence of an ongoing treatment for relevant co-morbidities (n = 1, Group A)
- Several participants reporting the urge to urinate before the end of the digestion period, preventing administration of neuromodulation (n = 3, n_{GroupA} = 1, n_{Groupb} = 2)

Unfortunately, exclusion of these participants did lower the statistical power of the analysis, resulting in a final final sample distribution of N_A = 14, N_B = 14, N_C = 15. The non-normality and relatively small sample size of this distribution required that non-frequentist statistical techniques be used.

Despite this, the results of the study were nonetheless promising. Analyzing the effects of the intervention, it was clear that there was a differential effect of stimulation frequency on urge-onset time. To account for the small sample size, and non-normality of the dataset, a Bayesian Robust Linear Regression was conducted, rather than a typical frequentist approach. Given the potential for outliers arising as a result of the sample size, a Student's t distribution was selected during likelihood calculation (N_{draws} = 12, 000). The model indicated that group A (low-frequency TTNS) had a mean time to first urge of 937 seconds ($HDI_{3\%}$ = 575.88s, $HDI_{97\%}$ = 1291.88s, see fig. 3A). Posterior distribution comparison indicated an 89.28% probability that individuals exposed to low-frequency TTNS experienced urge-onset more rapidly than placebo control (group B, *mean* = 1277.7s, $HDI_{3\%}$ = 880.68s, $HDI_{97\%}$ = 1648.17s). As detailed in fig. 3 , also indicated that group C (high-frequency TTNS) displayed a delayed urge onset compared to placebo control (*mean* = 2301.93s, $HDI_{3\%}$ = 1931.67, $HDI_{97\%}$ = 2642.83s) with posterior analysis indicating a 99.94% chance that high-frequency intervention delayed urge onset in individuals within group C, when compared to those in group B. All parameters indicated excellent model convergence ($\hat{R} = 1.00$). These results indicate the likely presence of a promising frequency-dependent trend, however additional analysis is critical as the sample size remains too small to make any certain claims at this stage.

Nevertheless, an additional Region of Practical Equivalence (ROPE) analysis was conducted to assess whether the difference in urge-onset time between groups A and B (which would indicate a novel effect of TTNS) were clinically relevant. A range of ±60s was selected as the region of practical equivalence for the analysis. Doing so revealed that 7.49% of the posterior distribution of the difference in urge onset fell within the region of equivalence indicating a considerable likelihood of potential clinical relevance (see Supplementary Materials)

TTNS Has Little Frequency-Dependent Effect on Perceived Urge Intensity

Of all experimental participants (including those removed from statistical analysis) only one individual declined the offer to take part in the washout period, resulting in a participation rate of 98%.

A factorial ANOVA suggested there was a significant main effect of the washout period ($F_{(1,79)}$ = 17.625, p < 0.00001) but not group allocation ($F_{(2,79)}$ = 1.276, p = 0.28) on urge intensity. In addition, there was no significant interaction between the factors reported ($F_{(2,79)}$ = 1.076, p = 0.35). In all cases, there was an increase in the average urge intensity reported by the participants before and

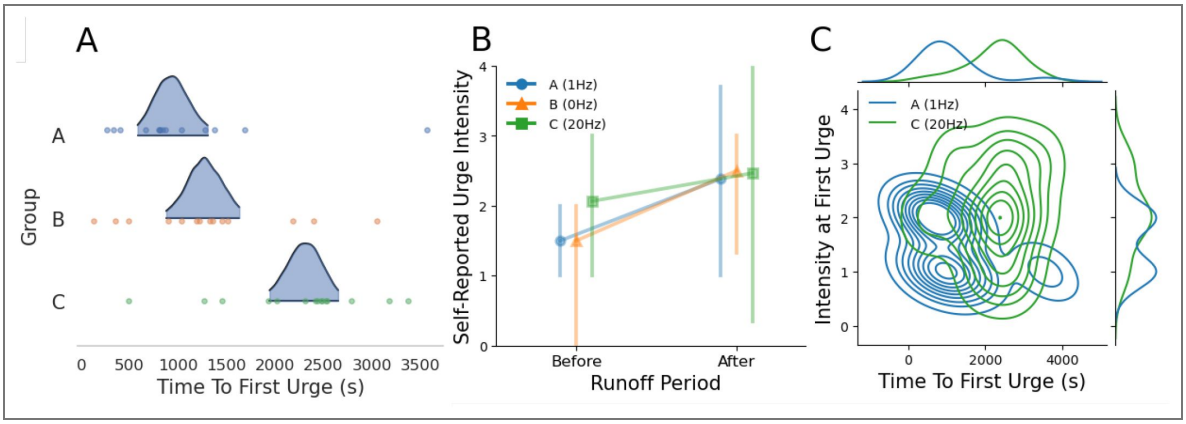


Figure 3. **Experimental Study Results**

A: effects of low (1 Hz, Group A), high (20 Hz, Group C), and placebo (0Hz, Group B) TTNS stimulation on the time elapsed to first sensation of urge. Shown are raw-data points and Bayesian posteriors for each group.**B**: effects of the washout period on self-reported urge intensity. Error-bars = ±95% Confidence Interval. Urge intensity self-reported on a scale of 0–4 (see methods), washout period was 10 minutes in duration. **C**: bivariate and univariate kernel-density estimate plot displaying the relationship between the time elapsed before participants reported the urge to urinate (i.e., the unit reported in panel A), against the self-reported intensity of this urge at (i.e., how strongly participants felt this initial urge, the data reported in panel B before the additional 10-minute washout period).

after the washout period (see Fig. 3B). At the end of the washout period, the three conditions finished with a similar intensity score. However, the variability within this data makes any robust inferences impossible.

There was a small but obsertable relationship between urge intensity and sensory response (i.e., time elapsed to urge sensation). While urge intensity at the first reporting point (i.e., before the washout period) was similar across groups, those in the excitatory condition reported the sensation earlier compared to those in the inhibitory condition (see Fig. 3C).

Normal Bladder Activity May be Computationally Modeled

Under baseline (unmodulated) conditions, the behavior of the model was promising. The model accurately simulated normal bladder function and the associated efferent nervous activity. Specifically, it was able to reproduce the expected filling and voiding cycle (see Fig. 4 top trace). During the filling phase, the efferent projections associated with urine storage (hypogastric and pudendal) were tonically active, while the pelvic projections associated with voiding were silent.

In contrast, during void events, bursts of pelvic efferent activity silenced the hypogastric and pudendal projections. This change in activity was associated with a decrease in bladder volume, indicative of a switch in the system from a storage state to voiding (see fig. 4). These results suggest that the model was able to mimic the complex switching behaviors required to mediate the behavior of the lower urinary tract, allowing it to serve as an accurate baseline upon which to test the impact of modulation.

Though the model does display the ability to accurately capture bladder-like behaviour it should be noted that there were several limitations that may limit its generalisability. The model displayed a much smaller bladder capacity (mean 112*ml*, ±11.6) than observed physiological values in humans (300 – 500*ml*[28]). Additionally, the model displayed poor voiding efficiency. Under normal (unmodulated) conditions the average voiding efficiency was 14.5% (±31.4%). The low average efficiency coupled with the relatively large variance limits the direct physiological comparisons that may be drawn from the model.

These limitations are likely due to the training data used during model fitting. The original dataset was obtained from male Wistar rats[24]. The average capacity across the dataset was 0.7*ml* (±0.09). Moreover, it also displayed a similarly poor voiding efficiency throughout (mean=9.54%, ±2.59) - likely due to the tightly constrained nature of the recorded bladder behaviour. While the reduced capacity and voiding efficiency of the training dataset were partially overcome (as the performance of our model outperformed in these metrics slightly) the limited generalisability of the model should be kept in mind when interpreting the results.

Despite this limited generalisability the model never nevertheless serves as a more than acceptable foundation for drawing proof-of-concept conclusions and examining potential mechanistic explanations for tibial nerve simulation.

Computational Modeling Confirms the Frequency-Dependent Effects of Tibial Nerve Stimulation

Analyzing the effects of TNS on the computational model revealed an intriguing trend. Under baseline conditions, the model produced a cycle of storage and voiding in accordance with expectations from previously obtained animal data (see [24], Fig. 5A). Applying high-frequency (20 Hz) stimulation to the system completely inhibited the voiding action (according to the expected effect of TNS on bladder behavior). However, if low-frequency (1 Hz) TNS was applied to the system, voiding occurred earlier (baseline mean=426*s*, ±14.6; low-frequency mean=410*s*, ±9.9) and with greater voiding efficiency (baseline mean=9.54%, ±2.59; low-frequency mean=41.44%, ±21.72) causing larger drops in stored volume for a given voiding event (see fig. 5A and supplementary materials).

To do so, the research team conducted a further exploration of the system during low-frequency TNS. To guide this exploration an initial hypothesis was generated. In theory, to drive any increase in voiding activity *without* considerably changing the time elapsed before voiding (i.e., the time of

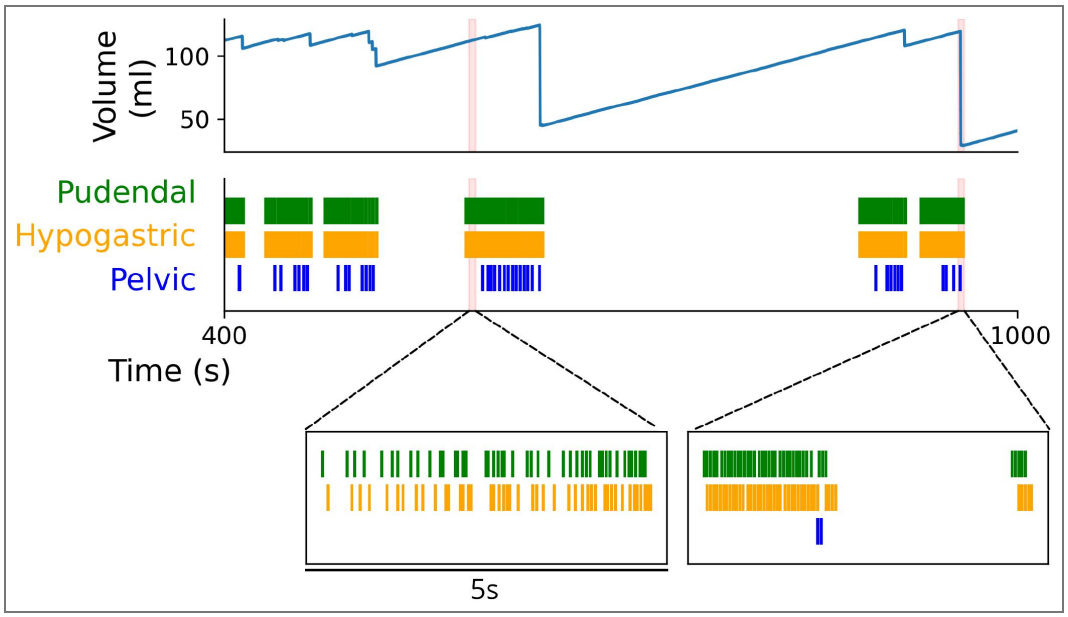


Figure 4. **Behavior of the Simulated Bladder Model.**

Shown is the bladder behavior (top trace) and associated efferent neuronal activity (middle trace) recorded from a 1000-second duration simulation. Blowout boxes contain 5 second windows of the full recorded activity (highlighted in red) during filling (left box) and voiding (right box). Each raster trace was obtained from a randomly selected neuron within the Pudendal, Hypogastric, and Pelvic units ($N_{neurons}$ = 100 in each case).

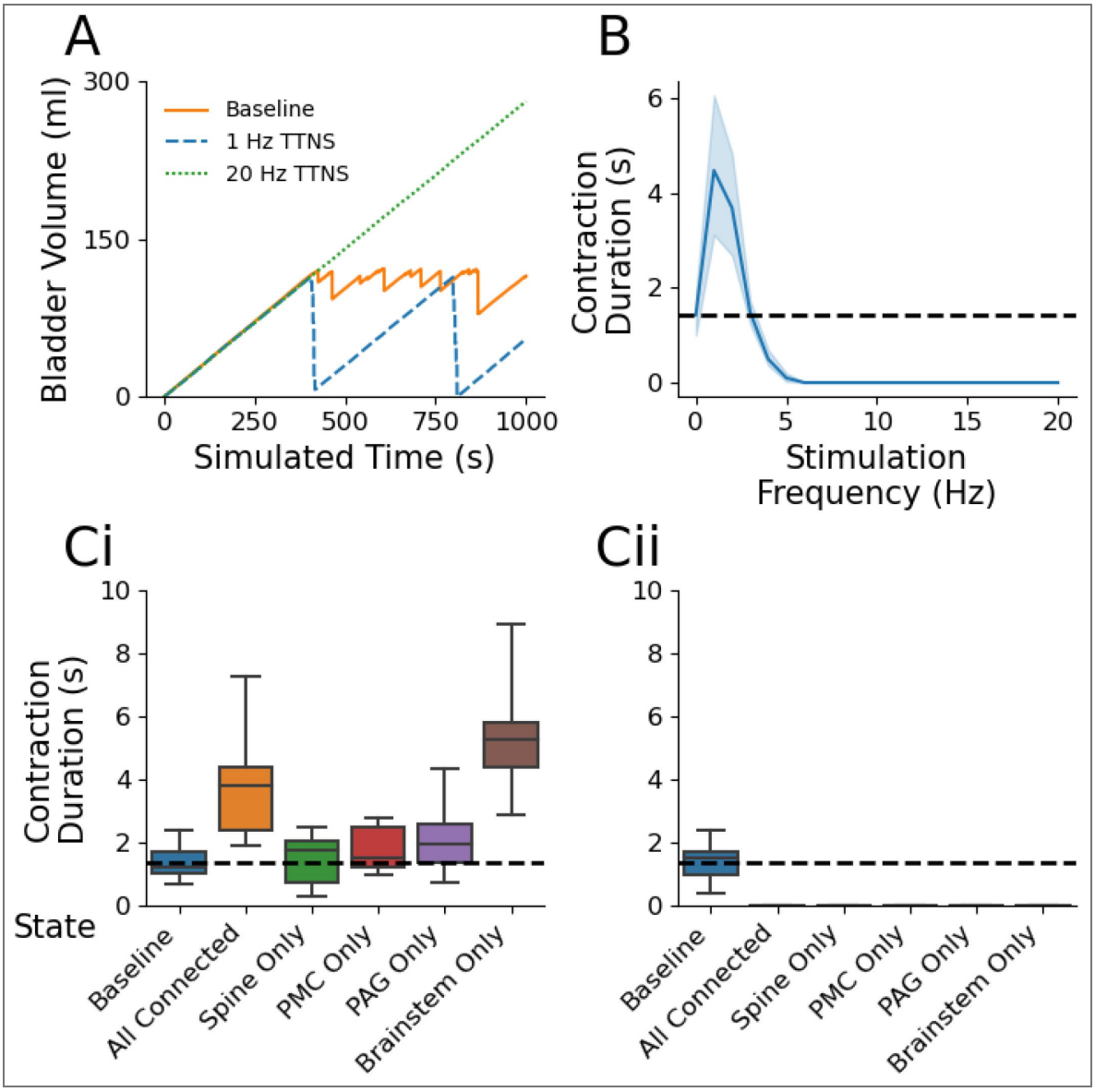


Figure 5. Computationally Modeling TTNS.

A. Effects of low (1 Hz) and high (20 Hz) frequency TTNS on simulated bladder function compared to unmodulated (control) conditions. **B**. Frequency dependent effects of TTNS on bladder contraction. Shown is the average total bladder contraction duration for a 500s simulated period under 21 different stimulation frequencies (0-20 Hz, in 1 Hz increments, $N_{repeats}$ = 10). Errorbar= ±95%*CI*. A contraction duration of zero ms indicates that voiding was completely inhibited (i.e., no voiding events occurred during the simulated period of time) **C**. Effects of disconnecting specific tibial-nerve projections on total contraction duration (for all voids over a 500s simulation period) under low-frequency (1 Hz, **i**) and high-frequency (20 Hz, **ii**) conditions. In both cases, baseline behavior represents the unmodulated behavior of the model (i.e., where all tibial-nerve projections remained intact *and no tibial-stimulation was applied*). In contrast, the "all connected" conditions describe a model configuration where all tibial-nerve projections remained intact *and with tibial-stimulation applied at 1 Hz (i) or 20 Hz (ii).* $N_{repeats}$ = 10 in each condition.

void-onset) one or more of the following changes may be occurring:

- The intensity of bladder contraction is increased
- The duration of bladder contraction is increased
- The system-level mechanisms that control urine storage are being inhibited.

Further analyzing the behavior of the system under a range of different stimulation conditions, the research team discovered a frequency-dependent relationship between TNS and contraction *duration*. For a given simulation, at low frequencies the average effective duration of contraction – i.e., bladder contractions that successfully induced a voiding event were increased significantly, while at high frequencies they were eliminated completely, resulting in a contraction duration of 0ms (as voiding became impossible, see fig. 5B). These findings confirm the presence of a frequency-dependent effect of TNS on bladder behavior and indicate that the low-frequency effect may be driven by the extension of bladder contraction, causing a greater intensity of voiding for a given simulation run.

Brainstem Targeting Projections Mediate Low-Frequency Excitatory Effects

As the duration of contraction was highlighted as a measure of interest by the initial computational analysis, we sought to understand the nature of this frequency-dependent effect by analyzing the specific projections that may mediate it. To do so, an analysis of bladder contraction was performed in a range of connective states (where specific tibial nerve projections were severed) during low-and high-frequency stimulation.

A one-way ANOVA revealed a significant effect of the connective state on the total duration of contractions (i.e., across all voiding events) over a 500s simulation during low-frequency ($F_{(5,54)}$ = 16.975, p < 0.0001) and high-frequency conditions ($F_{(5,54)}$ = 55.269, p < 0.0001).

From these results, it is clear that brainstem, rather than spinal cord, targeting projections are necessary for the manifestation of the low-frequency effect, though it cannot be said at this stage how each module contributes to the effect (as both were required to produce a large-magnitude increase in contraction duration). The effect size of the PAG only condition was larger than that of the PMC condition providing some evidence that this region may be playing a greater role in the process however further research is required to disentangle the individual effects of each region. Intriguingly it appears that the inclusion of spinal-targeting tibial projections has a pronounced influence on voiding efficiency, with the elimination of these projections (leaving only brainstem projections intact) causing a more consistent increase in voiding efficiency.

The Mechanism Underlying High-Frequency Inhibition Remains Unclear

In contrast, the combination of projections that underlie high frequency stimulation is more opaque. Bladder contractions were extinguished by any combination of spinal or brainstem targeting tibial projections when 20 Hz TNS was applied. (see Fig. 5Cii). This finding makes it difficult to pinpoint the exact mechanism that underlies high-frequency inhibition of bladder activity (as all tested configurations were able to induce the effect). These findings require further analysis, but nevertheless provide additional evidence for a clear inhibitory effect of high-frequency TNS on bladder function.

Discussion & Conclusion

Experimental Study Findings

The results of the present study demonstrate that TTNS is able to impart a frequency-dependent effect on bladder function. They corroborate preliminary animal data within the literature [16–18] and for the first time demonstrate evidence for a frequency dependence in humans. The results suggest that the main effect of the intervention on the healthy population was sensation. Changes in the time taken for participants to experience the urge to urinate were the primary change in

bladder behaviour, in contrast to the effects on urge intensity which were minimal, though significant variance within the data makes any conclusions difficult. Although 20 Hz stimulation appeared to increase the average intensity of the urge after treatment, this is unlikely to indicate the presence of any lingering effect. Rather, it is more likely that this difference compared to 1 Hz stimulation was due to more time elapsing before participants reported any feeling of urgency (see Fig. 3C). In other words, while a similar intensity was reported upon feeling the urge to urinate, the time taken to produce this feeling could be modulated - either reducing it (via 1 Hz stimulation), or increasing it (via 20 Hz stimulation). This result is surprising, as it would be expected than any modulation in one aspect of afferent bladder sensation (the urge onset) would reasonably drive a change in the other (the urge intensity). While this juxtaposition undoubtedly warrants further exploration it is possible to hypothesise a potential explanation of the effect. As will be shown by the results of the computational modeling it may be the case that any afferent sensory changes observed are secondary to the primary effect of TTNS - the modulation efferent bladder activity. TTNS has been shown to alter the activity of a number of regions of the brainstem and cortex associated with bladder function[29] in different ways dependent on bladder volume[30] suggesting a complex state-dependent sensory effect. Though not explored in the present publication it is possible these secondary effects (i.e., those outwith the PAG and PMC targets modelled here) influence the conscious perception of bladder sensation in a complex and temporally specific manner.

Computational Findings

The limitations of the pilot study are somewhat remedied by the results of the computational modeling work which provide considerable mechanistic context to the experimental study. The unmodulated behavior of the model was promising, suggesting that it was able to accurately mimic the complex switching behavior employed by the micturition control system[2]. The system produced tonic storage-related efferent activity indicative of a guarding reflex during periods of lower bladder volume which transitioned to void-promoting pelvic activation and silence of storage-related activity at appropriate times. The switch in the circuit state aligned with voiding events in the data. These results suggest the model is able to capture both aspects of micturition (upper circuit activity and lower-bladder behavior) providing an accurate overview of the system for further research. Although the average bladder capacity of the model was lower than would be expected *in vivo* (simulated capacity was approximately 100-150ml compared to a physiological range of 300-400ml [31]) the accuracy of the high-level behavior of the system allowed the effects of TTNS to be studied *in silico*. The impact of TTNS on the system was clear: By varying solely the frequency of stimulation, it was possible to up-or down-regulate simulated bladder activity. This was in line with the hypothesis stated in the article, confirming the presence of a frequency-dependent effect highlighted by the experimental study. Though these results are promising there are several key limitations to our computational approach that should be kept in mind when interpreting any results. As previously mentioned the model displayed a bladder capacity and voiding efficiency considerably lower than normal human physiological ranges. Though this may be explained by the training data used during the fitting process it nevertheless reduces the physiological generalizations that may be drawn from the model. Moreover, the model did not simulate nociceptive afferent projections. Therefore, at present it cannot be used to test the efficacy of TTNS on a pathologicaly active lower urinary tract system. As such, care should be taken when directly comparing the computational findings to biological reality as they cannot directly inform us of any therapeutic clinical effects. Rather the present findings should be viewed through the lens of a systems level proof-of-concept approach. Nevertheless, even with a relatively narrow system-level perspective, our results still provide further evidence and a potential mechanistic explanation for an under-explored excitatory effect. These findings are more than sufficient to serve as an initial foundation for future clinical work.

Joining the Two

Though these results are evidently promising, when the computational and human findings are taken together, they appear to paint different pictures of the frequency dependent effect. The pilot study indicates a primary effect of the intervention on afferent sensation (with urge sensation being the primary measure) while the computational model primarily indicates an efferent effect through a change in contraction duration (though there was a minor afferent effect present). Analysing the computational results, it is tempting to assume the primary effect of TTNS was on efferent behaviour as the change in contraction duration observed *in silico* was considerably greater than the equivalent shift in void onset time, mirroring the relatively small effect size seen in the pilot study where 1 Hz stimulation was found to induce the urge slightly earlier, but not at a considerably greater sensory magnitude. However, the relatively small sample size of the pilot study, and the simplified afferent model (which did not include nociceptive C-Fibres) preclude this conclusion at the present time.

The two measures are not unrelated. Both pertain to different aspects of bladder function, and as such further research must critically asses how the two outcome measures are connected, as the present work indicates that both may be affected by the TTNS in a frequency-dependent manner (though to varying degrees of functional efficacy). Nevertheless, these findings undeniably indicate to the presence of some form of complex frequency-dependent effect of TTNS on bladder related function

Clinical Relevance and Research Direction

The future clinical research directions of this frequency dependence are particularly important when considering the treatment of non-obstructive urinary retention (NOUR). Currently, the treatment of NOUR remains rather limited. Invasive sacral stimulation is the only approved neuromodulatory treatment option available to patients [32]. Previous attempts to use TTNS to treat NOUR have returned mixed results [33, 34]. However, this may be due to the use of inappropriate stimulation parameters. Previous research did not alter the stimulation parameters of the standard interventions used in the treatment of incontinence and, as such, may have in fact had a deleterious effect on NOUR symptomatology.

Moreover, at present, TTNS is typically applied to treat incontinence prophylactically, with weekly sessions promoting long-term improvement in symptomatology [35, 36]. However, these findings suggest that if TTNS is to be applied in the treatment of urinary retention, it may be more effective to administer the treatment in an acute manner, i.e., at the point of voiding. Doing so could up-regulate the bladder and facilitate unassisted voiding. If shown to be effective when implemented in this way, it could serve as an effective means of reducing the dependency of urinary retention patients on catheters, reducing the rates of urinary tract infections and improving overall quality of life [37, 38].

In addition to providing evidence for therapeutic value in the treatment of retention, the present work may also shed light on the mechanistic underpinnings of this frequency-dependent effect. Our results indicate that low-frequency excitation is mediated by brain stem-specific projections. However, this does not preclude an influence of spinal projections. We found a minor but observable influence of spinal cord projections on voiding efficiency. Keeping spinal projections intact during low-frequency stimulation, seemingly reduced the magnitude of the observed increases in voiding efficiency (when compared to the same experiment run with only brainstem projections in place). It cannot be said at this time what the specific interaction between spinal and brainstem projections but it is clear there is a complex relationship between the two which should be explored in future work.

Regardless of the interaction between the spinal and brainstem projections, it may be possible to characterise the clear role of the brainstem in low-frequency excitation from a system-level perspective by characterising the region as a filter. The inhibitory feedback mechanisms within the PAG and PMC (see Fig. 2 labels 1–4) allow the modules to act as a set of high-pass filters connected in series, preventing efferent void signaling until a sufficient afferent firing rate is

reached. Low-frequency TNS can induce an excitatory effect by inhibiting feedback mechanisms within the PAG, reducing the threshold of the filters, thus allowing a smaller afferent signal to pass through to the PMC (the primary micturion control region [2]) triggering void-promoting efferent activity.

While, inhibition of the PMC or PAG alone did not result in the same magnitude of excitation, this filtering hypothesis may explain the slight difference in both contraction duration and voiding efficiency between the two conditions. In both cases, contraction duration and voiding efficiency were greater when the PAG was targeted over the PMC. As the first filter in series (the PAG) it is possible that when left fully operational (i.e., where only intact PMC targeting tibial projections were active) it is capable of eliminating afferent activity before it can reach the PMC. In this configuration, the inhibitory state of the PMC matters little.

Although this filtering hypothesis effectively explains the relationship between spinal and brainstem projections during low-frequency TTNS, it cannot explain the complex interactions that may be at play during high-frequency TTNS. The results from the present study were unclear in this respect, as all conditions resulted in the same extinguishing of bladder behaviour making the formation of any explanation difficult. It is unclear if high-frequency inhibition occurs at the spinal level, silencing afferent activity before it reaches the filters (Fig. 2, label 5), or at the level of the brainstem, silencing communication between the modules that allows the production of void-promoting efferent activity (Fig. 2, labels 2–4) or even the efferent activity itself (Fig. 2, label 1). Preliminary evidence from the literature paints a picture of a complex relationship between spinal and brainstem tibial projections. Specifically, TTNS has been shown to have a directly inhibitory effect on spinal activity [8] that is nevertheless insufficient to induce bladder changes in cases of spinal cord injury [4]. Although this effect may be due to a rewiring of the bladder control system (as there is evidence that early application of TTNS has some effect [39]), coupled with the results from the present work it is clear there is a need for further research to explore the spinal and supraspinal aspects of the system interact to inhibit bladder function during high-frequency TTNS.

Future research should primarily focus on confirming if these system-level proof-of-concept results generalise from an *in silico* demonstration to real-world effects in a pathological population. Specifically, subsequent work should maintain a clear focus on exploring the clinical efficacy of low-frequency TTNS as a treatment option for urinary retention. Specifically, it would be prudent to determine if administration of the intervention in an acute manner, at a lower frequency, induces an objective, measurable change in bladder physiology. If followed through to completion, this research direction may remedy the mixed results seen previously. Additionally, these findings may contribute to the ongoing development of a wearable neuromodulatory treatment device which may provide a user-friendly method of administering the intervention [40].

Conclusion

Despite some limitations surrounding the methodology of the experimental study and the need for further research in this direction, the present findings nonetheless provide vital evidence for a frequency-dependent effect of TTNS in human beings. Moreover, they suggest a complex and not-necessarily solely brainstem-specific role of the tibial nerve in bringing about this unexpected, but critically important excitatory effect. We propose that these findings would lay the groundwork for a number of future research efforts which may elucidate the precise neural mechanisms underlying the complex effects of TNS on bladder function in both healthy and diseased states.

Supplementary Materials

ROPE Analysis

As part of the pilot study statistical analysis, a Region of Practical Equivalence test was conducted. A region of practical equivalence of ±60*s* was selected as an appropriate interval. Detailed in Supplementary fig. 4 is the result of the analysis, showcasing a 7.49% overlap in the distribution

of differences when comparing groups A, and B.

Bladder Urgency Survey

As part of the human study, participants were asked to rate how intense they felt the urge to urinate. This rating was adapted from a validated questionnaire typically used to rank urgency perception as a measure of urological dysfunction [21] (see Supplementary Fig. 1).

TTNS Stimulation Parameters and Example Electrode Placement

Shown in Supplementary Fig. 2 is a picture of the parameters set on the DS7A neurostimulator during the experiment. An example of the electrode placement may be seen in Supplementary Fig. 3.

Description of the Simulation Pipeline

Each run of the simulation began by specifying a total runtime (in seconds). At t_0, to introduce noise, the initial membrane potential of all neuronal units was randomized between the reversal potential for the leak-current (E_L, −80mV) and the threshold for action potential (V_{th}, −50mV). The bladder was assumed to be empty (V_B = 0) with an initial inflow rate determined via a stochastic process. At each time step (t), the bladder state was updated and the firing rates of the primary pressure sensitive afferents ($F(P_B(t))$) calculated. This firing rate was then used to update the status of the micturition control circuit and its efferent projections, which would be used to calculate the bladder state for (t + 1). The target values for the time step were then saved and the simulation increased to (t + 1). Upon reaching the final time step of the specified duration (t_{end}) any cached data were collated and exported, and the simulation stopped.

Synaptic Model

Synapses were modeled in terms of conductance using established formulae[42], modified to include opioidergic transmission. The magnitude of the synaptic current (I_{syn}) was defined as:

$$I_{syn} = g_{ex}\left(E_{ex} - v\right) + g_{in}\left(E_{in} - v\right) + g_{op}\left(E_{op} - v\right) \tag{5}$$

where $g_{ex/in}$ represents the conductance of classical excitatory/inhibitory currents, and $E_{ex/in}$ their reversal potentials; and g_{op}/E_{op} representing the action of opioid currents. Modeled synaptic conductance decayed exponentially according to

$$\frac{dg_{ex/in/op}}{dt} = \frac{-g_{ex/in/op}}{\tau_{ex/in/op}} \tag{6}$$

Synaptic strength was weighted, so that upon presynaptic spiking, the postsynaptic conductance of either excitatory, inhibitory, or opioidergic currents was incremented according to:

$$g_{ex/in/op} \mapsto g_{ex/in/op} + w\delta g_{ex/in/op} \tag{7}$$

where w represents the weighting factor, and $\delta g_{ex/in/op}$ the baseline increase in conductance in response to a presynaptic spike. The weights of all synaptic connections were determined by parameter fitting and constrained within a specific range to allow controlled plasticity within the network.

Computational Model Optimization

Bayesian optimization was used to fit the parameters of the model [43]. To do so, the input to the micturition control circuit had to be carefully controlled. To this end, for the optimization stage, rather than implement a noisy biophysical model, the bladder was represented as a mathematical vector that contained experimentally derived data on internal bladder pressure ($P_B(t)$) and

volume ($V_B(t)$). The sample rate of the vector was 50Hz, matching the model. The bladder data used to do so was obtained from previously published work[24] (see Animal Model Surgery and Preparation).

During optimization, the activity of second-order sensory afferents was extracted, downsampled to 10 Hz, and smoothed (1st-order Butterworth Filter, 1 Hz cutoff frequency). These data were compared to the ground-truth neural data associated with the input bladder data (see Animal Model Surgery and Preparation), producing a normalized root mean square error (NRMSE) value representing the difference between the simulated activity and expected activity. The algorithm adapted the weights and parameters of the model such that the NRMSE was minimized (acquisition function - Gaussian hedging, acquisition optimizer - LMBFGS, N_{calls} = 200). After running the algorithm, specific weights were manually adjusted to further optimize the efferent activity of the model, ensuring that it was accurate to typical bladder behavior.

Bayesian optimization of model weights and parameters considerably improved the accuracy of simulated afferent activity, bringing it closer to ground truth neural data ($NRMSE_{baseline}$=3.367; $NRMSE_{optimised}$=1.454). While subsequent manual tweaking did improve the efferent behavior of the model it did lead to a slight increase in NRMSE suggesting it did so at the cost of afferent accuracy ($NRMSE_{fitted}$ = 1.599). Despite the minor decrease in global function fit, this methodology nevertheless improved the efferent behavior of the model and allowed the simulation to better capture the peaking pattern of neuronal firing rates in the afferent arm of the circuit (see Supplementary Fig. 1) providing an accurate foundation for analyzing the effects of neuromodulation. Notably, the optimization algorithm reached its minimum NRMSE plateau in a reasonable number of calls suggesting the global function minimum was true (see Supplementary Fig. 7).

Although the optimized model did not capture every peak in the firing rate or the physiological noise present in the data, the current level of accuracy is more than sufficient to serve as a foundation for exploring TNS, as noise omission would likely only affect the model's ability to capture very specific network behaviors irrelevant to the present study.

Model Parameters

Where possible these were matched to the parameters described in the original papers describing the neuronal, synaptic, and lower-urinary tract models[23, 27, 42]. Key parameters that were fit included the current mediating tonic activity (I_{ap}), the magnitude of excitatory and inhibitory conductance ($g_{ex/in/op}$), and the upper magnitude of spike-frequency adaption ($\bar{g}_A$). See Supplementary Table 1 for the parameters used in the simulation.

Approved by the School of Informatics Ethics Board
(ID:977504)

Appendix B – Urge Intensity Survey (For Printing)

Urge Intensity Survey 1

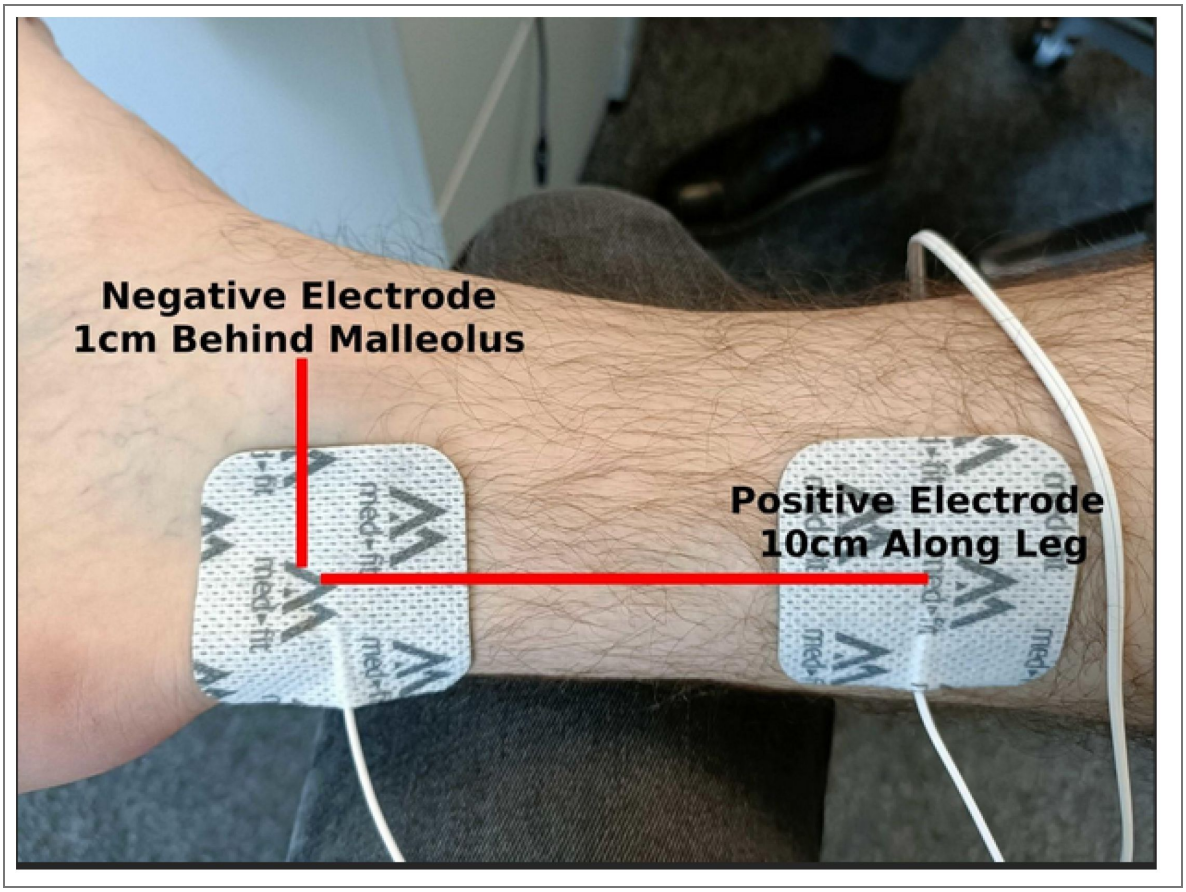
Please select one of the following options that best describes how intense your current urge to urinate is.

0 - Convenience (No Urge)	
1 – Mild Urge (Can Hold for Greater Than One Hour)	
2 – Moderate Urge (Can Hold for 10 – 60 Minutes)	
3 – Severe Urge (Can Hold for Less Than 10 Minutes)	
4 – Desperate Urge (Must Go Immediately)	

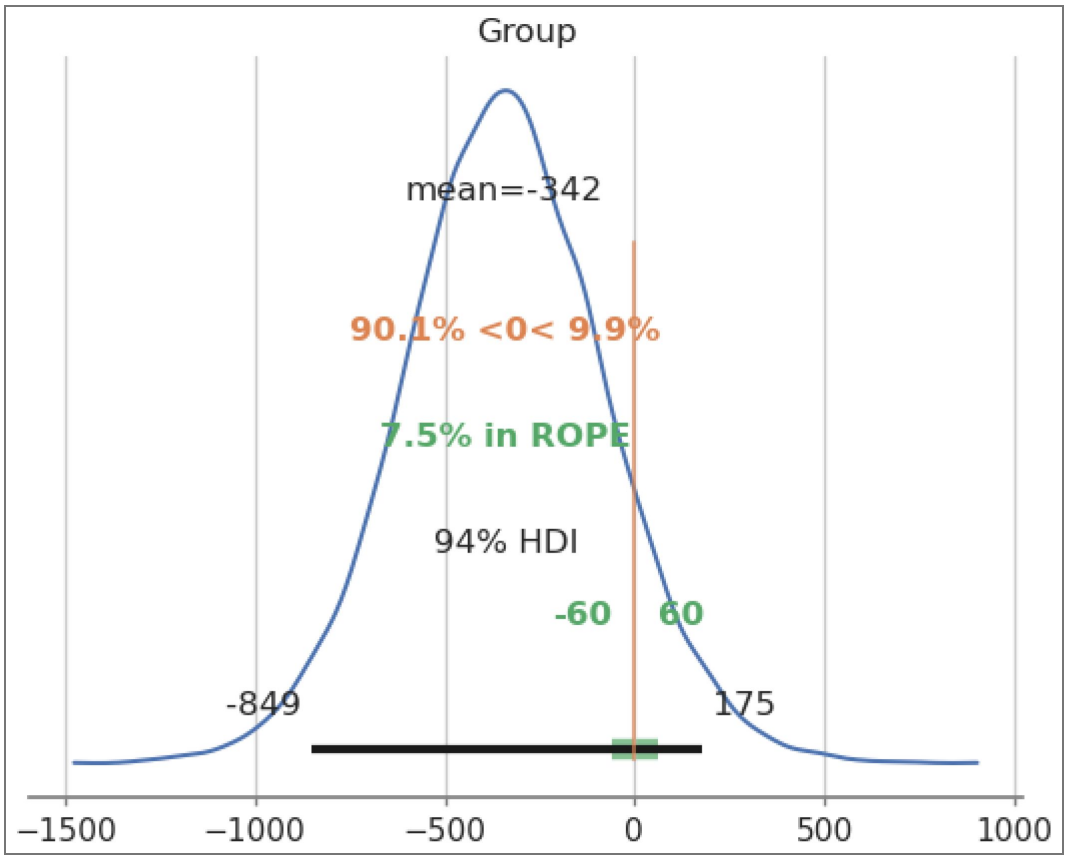
Supplementary Figure 1. Example urge-intensity survey. Shown is an example of the survey which was given to participants in printed form.

Supplementary Figure 2. Parameters Used During Neurostimulation

Shown is the configuration of the DS7A neurostimulator as part of the experimental protcol. Stimulation was monophasic, with a 200μs pulse width, max-voltage of 300V, with a stimulation frequency that was set using a timing module built in-house that could output a trigger signal at 1 or 20 Hz.

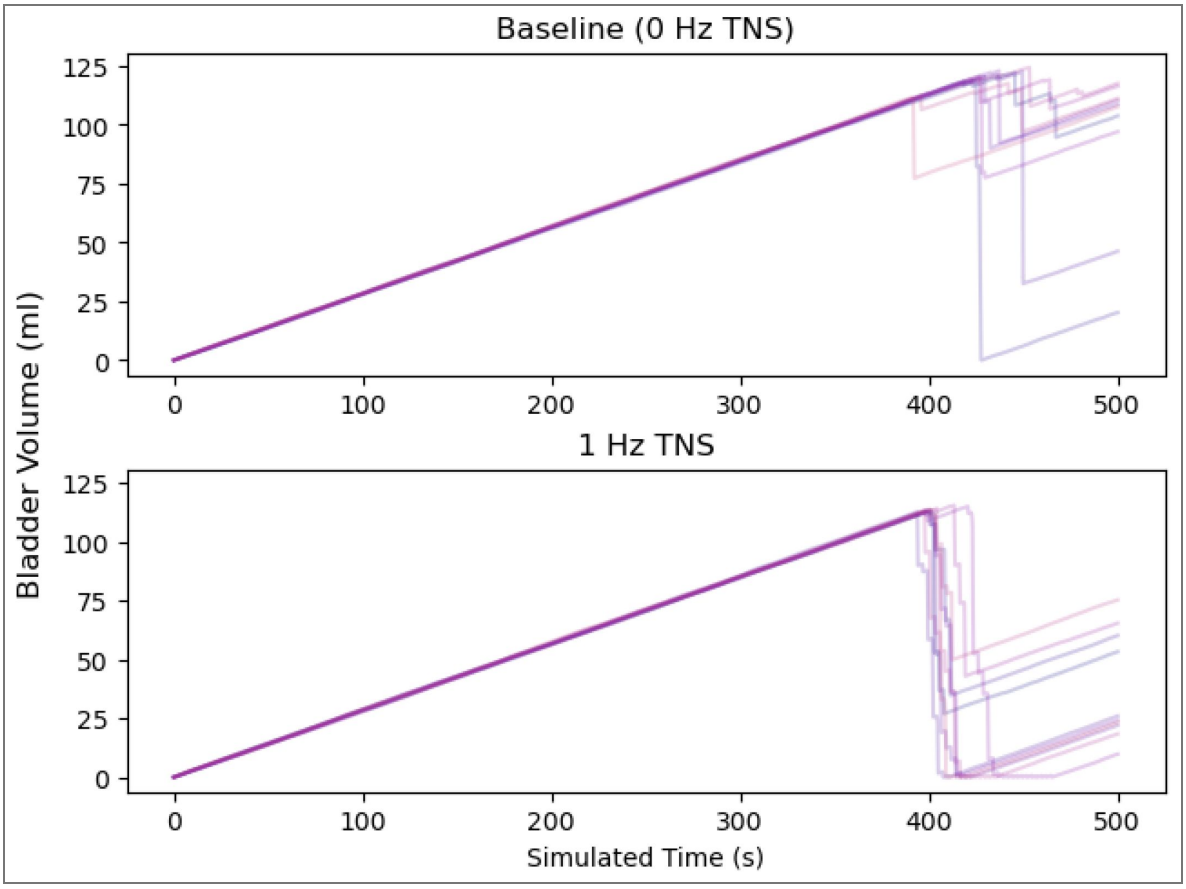


Supplementary Figure 3. **Standard Placement of TTNS Electrodes Used During Pilot Study**



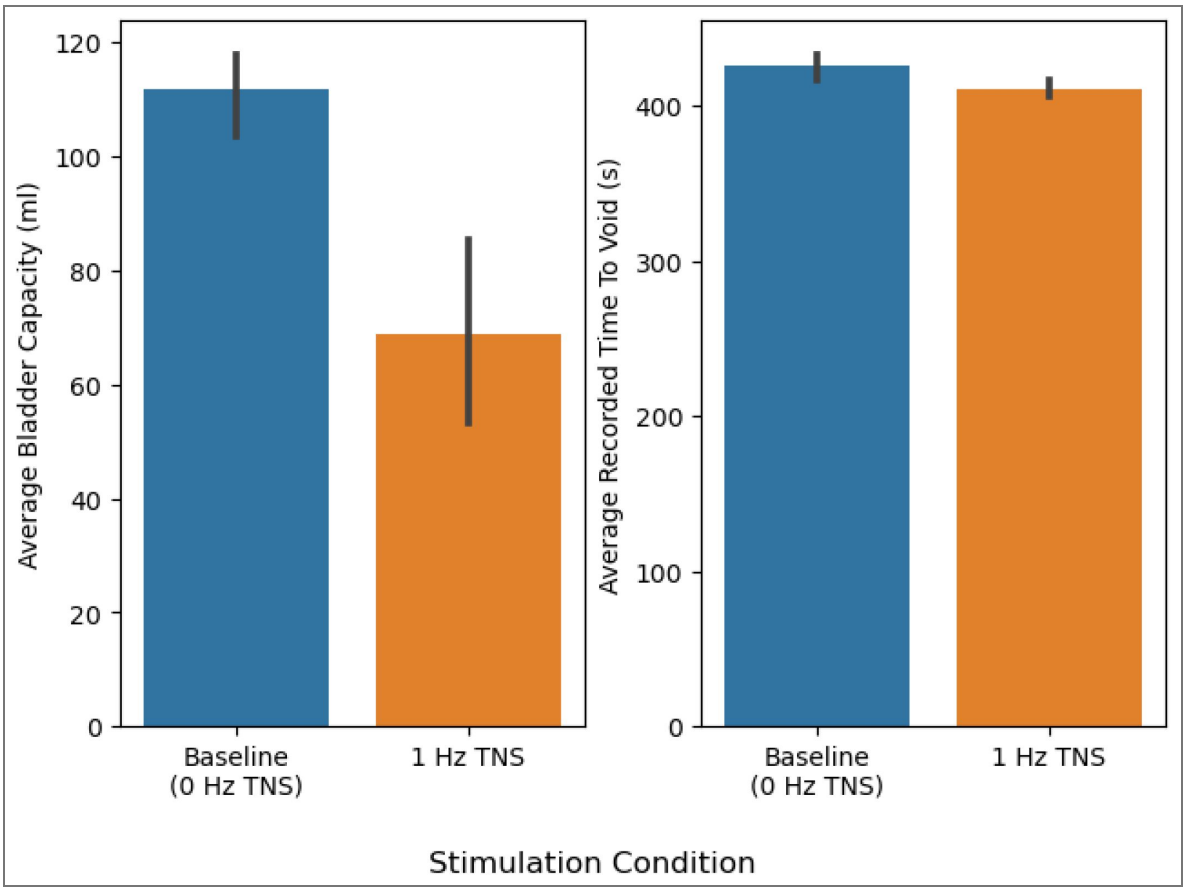
Supplementary Figure 4. Pilot Study Time To First Urge ROPE Analysis.

N_{draws} = 12, 000. Shown is the difference in time to first urge between groups A and B, in seconds.



Supplementary Figure 5. Simulated Bladder Volume Under Baseline and Low-Frequency Stimulation

Shown is the raw bladder volume from 10 independent bladder simulations (500s each) under baseline (0 Hz, top) and 1 Hz (bottom) tibial stimulation. All tibial nerve projections remained in-tact in both conditions.

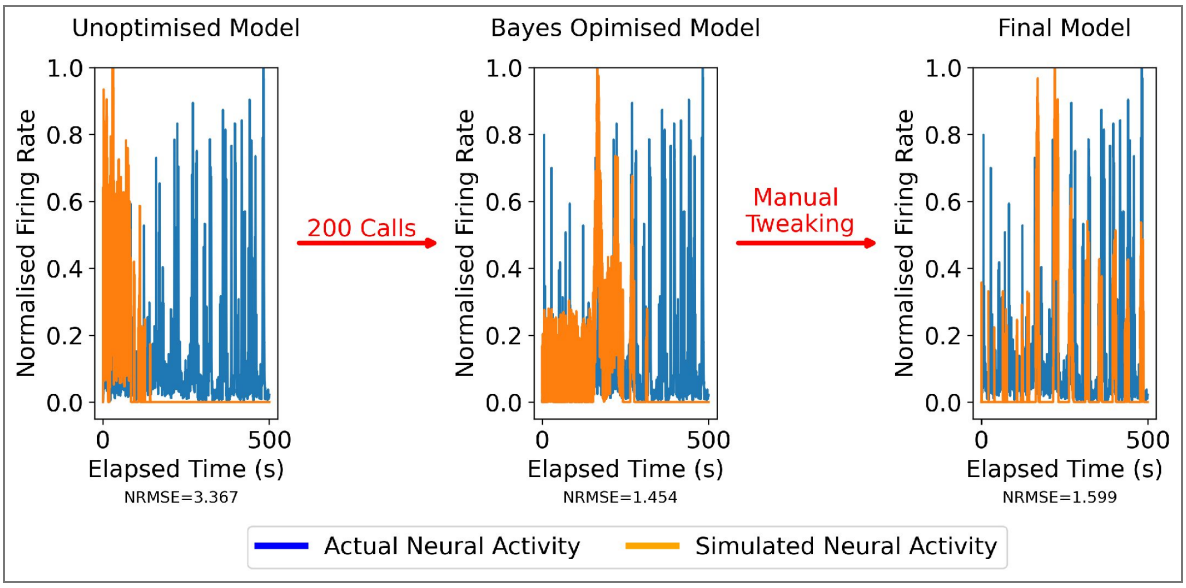


Supplementary Figure 6. Impact of TNS on Bladder Capacity and Void Onset

Shown is the mean bladder capacity at the time of voiding (left) and the mean simulated time elapsed before voiding commenced (right) from 10 independent bladder simulations (500s each) under baseline (0 Hz) and 1 Hz tibial stimulation. All tibial nerve projections remained in-tact in both conditions. Error bars = 95% CI.

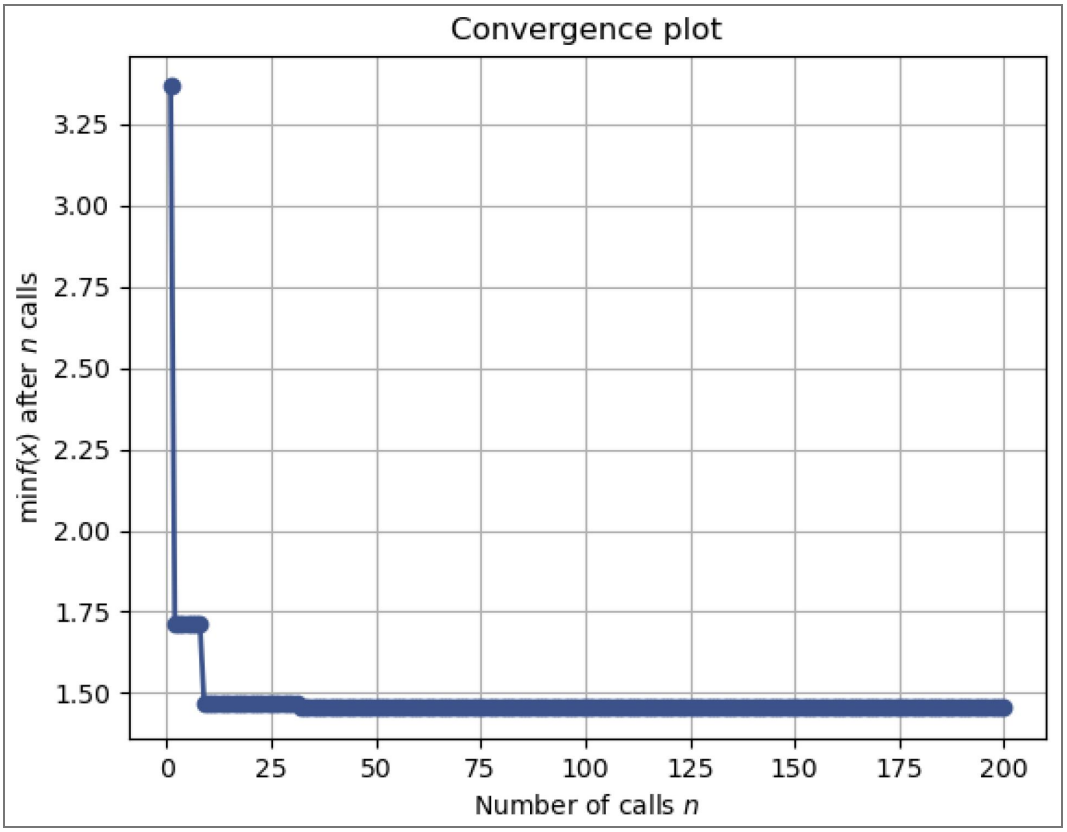
Supplementary Figure 7. **Model Performance During Fitting Process.**

Shown is the overlap between ground truth (blue) and simulated (orange) afferent neural activity for a subset of bladder pressure data. Model performance is shown in a random unoptimized state (left), after 200 rounds of Bayesian optimization (center), and after final manual optimization of model weights (right). The magnitude of the overlap between simulated and ground truth data at each stage shown as Normalized Root Mean Square Error (NRMSE). Smoothed firing rate (1st-order Butterworth filter, 1 Hz cutoff frequency) was normalized against the maximum recorded value in each dataset.



Supplementary Figure 8. **Bayesian Optimization Convergence Plot.**

Shown is a convergence plot detailing the Normalized Root Mean Square Error (NRMSE) of the ground truth vs. simulated afferent activity data at each phase of the optimization process.



Parameter	Value
Membrane Capacitance (C_m)	200pF
Tonic Activity Input Current (I_{ap})*	175.35pA
Leak Current Reversal Potential (E_L)	-60mV
Leak Current Reversal Potential - Tonically Active Neurons (E_{LTonic})	-70mV
Reversal Potential of Excitatory Current (E_{ex})	0mV
Reversal Potential of Inhibitory Current (E_{in})	-80mV
Reversal Potential of Opioidergic Current (E_{op})	-80mV
Reversal Potential of Adaption Current (E_A)	-70mV
Leak Current Increment (g_L)	10nS
Excitatory Current Increment (g_{ex})*	0.51nS
Inhibitory Current Increment (g_{in})*	1.40nS
Opioidergic Current Increment (δg_{op})*	1.5nS
Adaption Current Increment (δg_A)	1nS
Adaption Current Increment - Tonically Active Neurons (δg_{ATonic})	0nS
Maximum Adaption Current ($\bar{g}_A$)*	10.17nS
Maximum Adaption Current - Tonically Active Neurons ($\bar{g}_{ATonic}$)	2nS
Activation Threshold for Adaption Current (V_A)	-50mV
Activation Threshold for Adaption Current - Tonically Active Neurons (v_{ATonic})	-45mV
Adaption Current Slope (Δ_A)	5mV
Post-Spike Reset Potential (v_R)	-55mV
Spike Threshold (v_{th})	-50mV
Spike Initiation Slope (Δ_t)	10mV
Refractory Period (Δ_{tRef})	5ms
Opioidergic Current Decay Constant (τ_{op})	10ms
Excitatory Current Decay Constant (τ_{ex})	5ms
Inhibitory Current Decay Constant (τ_{in})	10ms
Neuroplastic Decay Constant (τ_{STDP})	20ms
Adaption Current Decay Constant - Tonically Active Neurons (τ_{ATonic})	40ms
Adaption Current Decay Constant (τ_A)	200ms
Synaptic Learning Rate	5×10^{-3}
Target Postsynaptic Firing Rate (p_o)	1Hz

Supplementary Table 1. Final Neuronal Model Parameters.

Where possible parameters were matched to the original specifications of the neuronal/synaptic model. Parameters that were altered by the model fitting process are marked as *.

Data availability

The custom code used for the analysis in this study is available from the linked GitHub repository (*https://github.com/Aidan-MT/TibialModulationSim*). The authors request that any individuals using the code as part of further research cite the present work. Raw data obtained from the pilot study experimentation will be available through the Edinburgh Data Centre.

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Additional information

Code availability

The custom code used for the analysis in this study is available at[41]. The authors request that any individuals using the code as part of further research cite the present work.

Author Contributions

A.M.T and K.N. conceived the study, A.M.T. constructed the computational model, and analyzed the results; M.J. and A.E. collected and processed the bladder data used to fit the computational model and run simulations in the present study; W.J. assisted with the recruitment and running of the study; E.L. created and provided the biophysical bladder simulation used in the computational model; A.M.T and M.J. produced the figures used in the publication; K.N. and S.M. provided research guidance and advice; All authors reviewed the manuscript.

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Peer reviews

Reviewer #1 (Public review):

Summary:

This manuscript examines the frequency-dependent effects of transcutaneous tibial nerve stimulation (TTNS) on bladder function in healthy volunteers, supported by a conductance-based computational model of lower urinary tract (LUT) neural circuitry. The authors show that 1 Hz TTNS modestly hastens the urge to void, while 20 Hz TTNS delays it - a finding with potential therapeutic relevance for underactive bladder (UAB). A computational model incorporating spinal, brainstem, and peripheral circuit elements provides a mechanistic framework suggesting brainstem-mediated pathways underlie these frequency-dependent effects. The revised manuscript addresses the majority of concerns raised in the initial review.

Strengths:

Novelty. Demonstrating a low-frequency excitatory effect of TTNS in humans is genuinely new. The possibility of inverting the therapeutic effect of an established neuromodulation intervention by simply adjusting stimulation frequency is clinically meaningful and opens a plausible treatment avenue for UAB.

Integrated approach. Combining a controlled human pilot study with a systems-level neural model is a notable strength. The model is physiologically grounded and serves well as a proof-of-concept tool for exploring mechanistic hypotheses.

Improved reproducibility. The addition of a public GitHub repository with documented code, supplementary figures detailing electrode placement and stimulation parameters, and removal of the externally derived Figure 3 all meaningfully improve transparency.

Improved statistics. The shift to Bayesian modelling with ROPE analysis is well-justified given the small sample size and more appropriate than frequentist testing in this context.

Improved presentation. Unit standardization, figure label corrections, and replacement of imprecise terminology (e.g., "paradoxical", "analytically") make the revised manuscript considerably clearer.

Remaining Concerns:

Afferent-efferent disconnect. The human study measures urgency (an afferent sensory endpoint), while the model's primary output is contraction duration (an efferent motor endpoint). The authors have added discussion of this mismatch, but should state more explicitly that the two lines of evidence are complementary rather than directly comparable, and that the mechanistic link between them remains a hypothesis.

Clinical contextualization of effect size. The excitatory effect of 1 Hz TTNS is modest. A brief reference to what a minimally clinically important difference might look like in UAB or urodynamics research would help readers gauge the translational significance of the finding.

Overall Appraisal:

The authors have achieved their stated aims: providing proof-of-concept human evidence for frequency-dependent TTNS effects and a plausible neural circuit explanation. The manuscript is now appropriately cautious in its claims. The open-source computational model is a useful community resource. This work is best understood as a well-scoped proof-of-concept study that credibly motivates further investigation.

https://doi.org/10.7554/eLife.106174.2.sa2

Reviewer #2 (Public review):

Strengths:

The main strength of the work is to call attention to a new possibility of inverting the effect of TNS in humans by manipulating stimulation frequency, opening new indications for the therapy. This is highly relevant because of the recent popularity of TNS and its non-invasiveness, which lends itself to rapid testing and evaluation for new conditions and high willingness to adopt. The authors convincingly demonstrate a modest excitatory effect on bladder sensation with low-frequency TNS, which clearly warrants further investigation.

The high-level design of the hypotheses, concepts, and experiments are clearly articulated in both the methods and in particularly clear diagrams, letting the reader focus their attention on the most important findings.

It is rare to develop a new computational model of the lower urinary tract at a systems level, and even more so for it to incorporate circuits in the spinal cord and brainstem centers, and this work undoubtedly advances the field's ability to engineer such systems. Further, because the model is comprised of linked conductance-based point-neurons, it is an excellent tool to investigate how an arguably plausible wiring diagram for neural control of the LUT could result in stimulation frequency dependent effects on pelvic efferents. It is a proof of concept demonstrating how their mechanistic hypothesis of TNS could be implemented neurophysiologically by the nervous system. Further, the model is shared openly, which conforms to good modeling practices.

Weaknesses:

The main drawback of the work is the overinterpretation of the results. The human study and computational model are both proof-of-principle. The human study effect size is small and the sample size is modest; the computational model is poorly validated and does not generate physiologically typical urodynamic responses when simulating even healthy nominal LUT conditions. Thus, both the existence of a TNS 1Hz inhibitory effect (human study) and the mechanistic interpretation of its origin (simulations) remain provisional. For example, despite some caveats later in the work, the abstract stating there is a "frequency-dependent effect of TNS via the ability to alter urge perception and down-regulate bladder activity, corroborating model predictions," could easily be misleading, since a) the reduction in time of first urge with 1Hz stimulation was quite small relative to overall void time, b) reported

intensity was essentially not impacted, and c) the model does not directly make predictions about these experiment outcome measures. Similar overreaching statements appear in the second to last paragraph of the introduction, the first paragraph of the discussion, and so on throughout the paper. Many of the analyses are bespoke to the idiosyncrasies of the dataset rather than field standards, making spurious results also more likely and the effects provisional. One example is the use of robust linear regression to identify significance in the experiment between the 1Hz and control groups AND removing outliers before the analysis, since the typical approach is to use robust regression when the outliers are left in the data. Taken together, the potential excitatory effect and mechanism are interesting, and perhaps worth further investigation, but are considerably more tentative than stated.

It remains ambiguous whether a TNS excitatory effect size shown (even if it ends up being repeatable) is clinically meaningful. The ROPE analysis is a reasonable start, but no attempt to connect the parameters chosen (e.g. 60s) to clinical outcomes were made. This is especially true given the washout results and lack of effect on perceived urgency.

There remain several reasons to treat the model results questionable. First, as the authors now note, the model under normal conditions does not generate normal function; a voiding efficiency of 15% is severely underactive. Second, the 1 Hz stimulation simulation appears to create normal voiding, suggesting that the implementation of the neural control circuits may not produce results that would generalize to other experiments. Third, analysis focuses on the model outcome of "time to void", but this outcome is not reported for the experiment, so direct comparison is not possible.

https://doi.org/10.7554/eLife.106174.2.sa1

Author response:

The following is the authors' response to the original reviews.

> ***Public Reviews:***
>
> ***Reviewer #1 (Public review):***
>
> *Summary:*
>
> *The research investigates the frequency-dependent effects of transcutaneous tibial nerve stimulation (TTNS) on bladder function in healthy humans and via a computational model. The authors report that low-frequency (1 Hz) TTNS accelerates the urge to void, while highfrequency (20 Hz) TTNS delays it, corroborated by a computational model suggesting brainstem-mediated mechanisms. The work bridges experimental and theoretical approaches to propose a novel framework for TTNS applications in urinary retention.*
>
> *Strengths:*
>
> *(1) The integration of human experiments and computational modeling is a major strength. The model successfully replicates bladder dynamics and provides mechanistic insights into frequency-dependent effects.*
>
> *(2) Identifies potential therapeutic applications for urinary retention, a condition with limited non-invasive treatments.*
>
> *(3) Figures are clear and illustrative, and supplementary materials provide essential methodological depth.*
>
> *(4) Controlled experimental design (eg., single-blinded, fluid/caffeine restrictions, etc), detailed computational model parameters and validation against animal data,*

> *transparency in data exclusion criteria and statistical adjustments.*
>
> *Weaknesses:*
>
> *(1) The study uses healthy participants; extrapolation to clinical populations (e.g., urinary retention patients) requires validation.*

The authors have included a statement noting this and explaining that future work will explore this.

> *(2) The simulated bladder capacity (100-150 mL) is lower than physiological ranges (300400 mL). While the authors note this, the impact on model validity should be further addressed.*

The authors acknowledge that the simulated bladder capacity and voiding efficiency of the model are lower than human physiological ranges. They have added an additional explanatory paragraph detailing this limitation and proposing the animal training data as a possible cause. Despite these limitations we do not believe this prevents the model from being used to explore proof-of-concept hypotheses (e.g., presence of frequency dependence, potential mechanistic bases) as in the present paper.

> *(3) The model omits nociceptive afferents, limiting its applicability to pathological conditions like overactive bladder.*

The authors acknowledge that this is a limitation of the model, and have included a paragraph in the paper's discussion detailing the limited scope of our *in silico* approach and clarifying the extent to which the results may be interpreted.

> *(4) The lack of significant differences in urge intensity between groups (despite timing differences) warrants deeper discussion. Is the primary effect on efferent activity (as suggested) rather than sensory perception?*

The authors acknowledge that this is a surprising result and as such have deepened the discussion of the pilot study results, including hypothesizing as to potential explanations and suggesting further research in the area.

> *(5) One of the highlights of this study is the identification of the effect of low-frequency (1 Hz) tibial nerve stimulation (TNS) on facilitating bladder contraction. Although the authors have clarified this effect in healthy participants, it would strengthen the conclusion if a UAB animal model (e.g., PMCID: PMC7927909, PMC8163611, PMC7847056, PMC8799394) were used to evaluate the same effect.*

The use of animal models is out with the scope of this study which aimed to act as a proof of concept work using a primarily computational approach backed by preliminary human data. The authors acknowledge that this does limit the strength of the conclusions. However, several animal models have been utilized in previous work (as cited in the publication) that demonstrate an excitatory effect of low-frequency tibial nerve stimulation. This work builds upon these previous studies to strengthen the case for a frequency dependent effect of the intervention.

> ***Reviewer #2 (Public review):***
>
> *Summary:*
>
> *Tibial nerve (electrical) stimulation (TNS) has emerged over the past 15 years as a non-invasive method to treat bladder overactivity, but interestingly, new animal work has suggested that TNS could actually be used to excite the bladder when appropriately tuning the stimulation frequency, effectively inverting its effect, perhaps opening the*

door to treat different conditions (e.g., UAB). The present study tests how healthy people respond to low and high frequency TNS, with the authors showing that they can substantially delay people's first sensation of bladder fullness with high frequencies (20Hz, shown many times before) but also that they can slightly hasten people's first sensation with low frequencies (1Hz, new result in humans). Moreover, the authors develop a computational model of interconnected conductance-based simulated neurons arranged in a physiologically plausible circuit that reproduces some aspects of the frequency-dependent effects of TNS. Their simulations suggest that we might expect low-frequency TNS to also increase the duration of bladder contractions in humans. The study highlights a potential new research direction, optimizing TNS stimulation parameters to increase basal bladder excitability.

Strengths:

The main strength of the work is to call attention to a new possibility of inverting the effect of TNS in humans by manipulating stimulation frequency, opening new indications for the therapy. This is highly relevant because of the recent popularity of TNS and its non-invasiveness, which lends itself to rapid testing and evaluation for new conditions and a high willingness to adopt. The authors convincingly demonstrate a modest excitatory effect on bladder sensation with low-frequency TNS, which clearly warrants further investigation.

The high-level design of the hypotheses, concepts, and experiments is clearly articulated in both the methods and in particularly clear diagrams, letting the reader focus their attention on the most important findings.

It is rare to develop a new computational model of the lower urinary tract at a systems level, and even more so for it to incorporate circuits in the spinal cord and brainstem centers, and this work undoubtedly advances the field's ability to engineer such systems. Further, because the model is comprised of linked conductance-based point-neurons, it is an excellent tool to investigate how an arguably plausible wiring diagram for neural control of the LUT could result in stimulation frequency-dependent effects on pelvic efferents. It is a proof of concept demonstrating how their mechanistic hypothesis of TNS could be implemented neurophysiologically by the nervous system.

Weaknesses:

The main drawback of the work is the frequent over-interpretation of the results. The human study and computational model are both proof-of-principle studies because the experimental effect size and sample size are modest, and the computational model is poorly validated and does not generate physiologically typical cystometric responses in simulations that are designed to recapitulate nominal LUT behavior.

Despite the stated caveats about the small effect in the human study, it should be emphasized throughout that this result is most reasonably interpreted as showing the possibility that TNS can have a low-frequency excitatory effect that merits follow-up, rather than a conclusive demonstration. The effect size is small (as the authors note) and should be placed in context with some minimally clinically important difference, if possible. The result is statistically significant, but even this may be subject to revision due to the small sample and the effect of post-hoc outlier removal and data analysis choices.

Acknowledged, the authors have included caveats in the discussion making clear that the present results should be interpreted as a proof of concept rather than a definitive demonstration. We note that in combination with existing animal findings these results strengthen the case for the existence of an unexplored excitatory effect of TTNS in human beings that may have valuable clinical implications if generalised.

> *Given the apparent mismatch between the model and the cystometric behavior at the systems level in the "normal" case (e.g., low capacity, low voiding efficiency, omitted pressure profiles, frequency, etc.) and the absence of quantitative model validation (e.g., it was not compared directly with any experimental data from human urodynamics or rodent cystometry, beyond the initial fit to the neural data, no sensitivity analyses were performed, no goodness of fit computed, etc.) the discussion should be much more circumspect about interpreting the results at a systems level and should probably contain a paragraph explicitly detailing the limitations of the model. The subsequent interpretation should focus narrowly on the neural circuitry, rather than things like contraction duration, where the model is at its strongest. As written, the authors over-interpret what the in silico study can reasonably be used to infer about LUT function.*

The authors have reworded the discussion section, including a limitations paragraph containing caveats about the interpretation of the results. We make clear that a systemslevel perspective should be maintained and that futher research is required to validate and generalise these results.

> *More justification is needed for why the contraction duration of the model is the central focus of analysis, when it connects only tentatively to the human study results, which focus on urgency. While not necessarily incorrect, a clearer link or motivation should be offered for how this informs our understanding of frequency-dependent TNS afferent or efferent inhibition during filling (which was the focus of the human studies and the abstract). In other words, why doesn't the model reproduce the 1Hz excitation effect of expediting void onset (or urgency in the human study), and why is it justified to look at contraction duration as a surrogate measure?*

The authors acknowledge this issue, and have included an additional section to the discussion considering the disparity between afferent and efferent effects observed across the pilot study and computational experimentation. The need for further research within this area to disentangle the complex nature of the frequency dependence has been stressed.

> *The authors claim that "voiding behavior occurred earlier [at 1Hz stim in the model]", pointing to Figure 6A as evidence, but this panel appears to show a single example model run where 1Hz voiding occurs only ~1s earlier (display makes this very hard to estimate). This is insufficient evidence to support the claim. Later, it is stated that "TNS did not ... void much earlier". The claims should be made compatible, and all such claims should have reasonable supporting evidence.*

The authors have included additional information in the supplementary materials to support the claim.

This information includes the bladder volume profile of a number of simulations under 0Hz and 1Hz conditions as well as the average void-onset time (i.e., simulated time before first void).

> *There are a number of reporting concerns that can be easily addressed:*
>
> *(1) Human Study:*
>
> *(a) To interpret the human study analysis, a fuller description of the "optional 10m inute extension" is necessary. How were participants presented with this option, how was blinding preserved, what fraction of participants accepted, and did phase 1 results influence their decisions to continue?*

The authors have included additional clarification detailing how blinding was maintained during the washout period. Additionally, we have included a section in the results which

details participation rates for the washout period. Given that only one participant declined participation in the washout period we do not believe it is necessary to conduct an analysis on what factors influenced participation.

> *(b) For reproducibility, details about the TNS parameters should be articulated, such as the method of determining "motor thresholds" (unless this is synonymous with "urge to urinate"), the shape of the stimulation pulses (e.g., biphasic, charge balanced), typical applied current, etc.*

The authors have included the requested information and added two figures to the supplementary materials detailing the parameters of the equipment and the exact electrode placement used during the pilot study.

> *(2) The Computational Model*
>
> *(a) The code availability statement for this type of work is inadequate. The model used for simulations in this work, as well as the code used to initialize (and randomize synaptic connections), needs to be hosted publicly because i) a model this intricate is extremely hard to reproduce/verify without code, ii) simulations are an essential piece of the argument, iii) hosting code requires very little overhead. Although there is an appropriate level of detail in the model description, it would not be possible to reproduce the model in any reasonable amount of time (or at all) because of the implementation-level details that are, understandably, omitted from the methods (e.g., what is a "unit", what 'exactly' do the connections in the PMC and PAG diagrams relate to, what were the final parameters used for all conductances, which parameters were "matched" to the original papers and which were not, etc.).*

The authors have included a link to a public GitHub repository where any interested individuals may download and use the code on their own machines for their own purposes. The repository, which includes a readme file detailing the operation of the model, as well as the thoroughly documented code provide the necessary transparency as suggested by the reviewers. We hope that by making the code open-source in this manner further research efforts by any interested researchers will be stimulated.

> *(b) Critical cystometric/urodynamic values that are typically analyzed to assess healthy LUT function are detrusor pressure (timeseries) and/or post-void residual or voiding efficiency (scalars). These should be included to verify that the model is representative of the "normal" case. This is especially important because the model's "normal" behavior appears to have extremely low voiding efficiency (Figure 6A).*

The authors acknowledge this limitation and as such have modified the simulation files to calculate and return: detrusor pressure, post-void residual, bladder capacity, and voiding efficiency (calculated post-hoc from these values). It should be noted however, that implementing this change required that the computational results be re-run using the new code. As such, the exact details of Figure 5 now differ slightly (though the high-level results and implications remain unchanged).

While the high-level results surrounding the frequency-dependence of TTNS and the likely brainstem specific cause of this effect remain unchanged, there were minor changes in the results of the computational projection experiments that necessitated a re-write of a portion of the results section.

Additionally, the authors have added a section exploring the low-voiding efficiency of the model at baseline and potential explanatory factors.

> ***Recommendations for the authors:***

> ***Reviewer #1 (Recommendations for the authors):***
>
> *(1) In Figure 6Cii, the high frequency is labeled as 10 Hz, but it should be 20 Hz. The authors should correct this in the figure legend.*

Acknowledged, the typo has been corrected.

> ***Reviewer #2 (Recommendations for the authors):***
>
> *(1) Data and Analysis:*
>
> *(a) Greater detail on analysis exclusion is warranted. What does it mean to have "greater than normal water intake"? Why was a large "urge duration" grounds for exclusion? Was its threshold set post-hoc, which group was that participant from, and does its inclusion (or not) affect the results of the analysis substantially?*

The authors acknowledge the issue of data removal. As such, to address this limitation an alternative analysis was conducted. Rather than frequentist methods, a Bayesian modelling approach and post-hoc ROPE analysis was conducted which included a greater proportion of the dataset (excluding only those who did not undergo neuromodulation, or who directly met the exclusion criteria for the study). This approach was taken as bayesian methods are better suited for smaller sample sizes such as the one utilised in the present work. The ROPE analysis provides additional evidence for a real-world relevance of the effect on bladder function. Though the authors acknowledge that these results are preliminary they hope they will provide initial evidence for the translation of a novel effect of TTNS into human participants.

> *(b) It is my understanding that Figure 4C is a plot of G1Hz and G20Hz on the horizontal from 4A and G1Hz and G20Hz on the vertical from 4B-"before". Hopefully, this is correct, and perhaps there is some way to state more simply what data are being reported, as it took me some time to understand.*

The authors confirm that figure 4C is a representation of data from figure panels A, and B. Thee horizontal axis represents the temporal “"urge onset” and the vertical axis the subjective intensity experienced at this point. To clarify this, the authors adjusted the axis labels to make clear the data being reported. Additional clarification was also added to the figure legend.

> *(c) The choice of units in Figure 6 makes interpretation harder than it needs to be. Although not SI units, the field commonly reports volume in ml and duration in seconds or minutes (certainly not ms). The horizontal on Figure 6A is especially confusing, since sim cycles are not clearly defined, nor is the reason for the 20ms of them, or if the 1000s of total simulation time means compute-time or simulated time. Is Figure 6A (20ms/cyc) (50000cyc)(1s/1000ms)*(1min/60s) = 16.67 min of simulated time? If so, does the model show >6 voiding events in that time under normal conditions (which probably requires some explanation, since that is unusual)? Later (L216), other terminology of "simulation run" is introduced and further complicates the interpretation of how much simulated time is passing.*

Acknowledged, the authors have updated the units used in figures througout the publication to match standard SI notation (Fig 4: M^3 -> ml, Fig. 5A:M^3 -> ml, 20ms cycles -> seconds, ms->seconds). Authors have also updated the language used in the figure and the paper to make clear that the figure is referring to 500 seconds (16.67 mins) of simulated time.

> *(d) It appears that in Figure 6B that a contraction duration of 0ms means no contraction at all - unclear if that is also true for everything below the horizontal dashed line.*

> *(e) Using p-values for analyzing differences between average model outputs (Figure 6C) is not appropriate, since one can run the model as many times as needed, making any negligible effect size statistically significant.*

The authors acknowledge that the computational nature of the second analysis limits the statistical tests that may be reasonably applied. As such, they have rewritten the results and discussion section to instead compare mean differences/effect sizes without reliance on p-values specifically.

> *(2) Clarity and Presentation:*
>
> *(a) Figure 3 should be removed since it describes an experiment not conducted in this study and whose data was used only for model fitting, not an integral component of the model concept, analysis, or results. A short description and a paper reference are sufficient.*

The authors acknowledge this feedback and have removed Figure 3 from the publication. We have instead provided a reference and brief description of the data used to fit the parameters of the model.

> *(b) L46, based on my understanding, should read something like "...may be a frequency dependent of TTNS, where low frequencies up-regulate bladder activity while higher frequencies downregulate it."*

Acknowledged, this section has been reworded to improve clarity.

> *(c) Generally speaking, there is nothing "paradoxical" about a frequency-dependent response to e-stim, which happens throughout the nervous system and even in the LUT with pudendal sensory stimulation. "Surprising", "useful", "underexplored", etc., are all closer to the authors' meaning.*

Acknowledged, the authors have avoided the use of the term paradoxical to better represent the original intent of the research findings.

> *(d) I am used to "washout" rather than "runoff", but this is a journal style decision, and either is fine.*

Acknowledged, the authors have replaced the use of the term runoff with washout and adjusted figure 1 to reflect this change.

> *(e) L51 "analytically" is a mathematical keyword reserved for closed-form solutions, which is not what the authors actually refer to. Something like "computationally" or "in silico" is closer to their meaning.*

Acknowledged

> *(f) L172 "abnormality" should be "non-normality".*

Acknowledged

> *(g) L148 "Like the original model", presumably referring to Gorski?*

Correct, wording has been changed to make this clear.

> *(h) L208-220 Unclear precisely what is meant by "intensity of the voiding events" or "temporal nature of the cycle".*

Acknowledged, the authors have provided additional clarification to avoid confusion.

> *(i) Figure 6C Is "baseline" the nominal model without stimulation, while the "all connected" is the nominal model with stimulation? And all the rest of the conditions indicate what was cut in silico?*

Acknowledged, authors have reworded the figure legend to improve clarity.

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