Challenges in modeling the neural control of LUT

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1. Overview

The lower urinary tract (LUT) in mammals consists of the urinary bladder, external urethral sphincter (EUS) and the urethra. Control of the LUT is achieved via a neural circuit which integrates two distinct components. One component of the neural circuit is 'reflexive' in that it relies solely on input from sensory neurons in the bladder and urethra that is fed back via spinal neurons to the LUT. The second neural component is termed 'top-down' and is a conditioned input that comes from structures such as Pontine Micturition center (PMC), and Pontine storage center (PSC), that also receive the afferent sensory input from the LUT relayed through periaqueductal gray (PAG). The reader is referred to excellent references such as [1-3] from de Groat's group for the top down control, which is not discussed here. This chapter focuses primarily on outlining the challenges in the development of computational model of the neural circuit that controls the LUT. Section 2 describes the anatomy of the LUT with primary focus on the neural anatomy. We describe the overall efferent and afferent neural pathways of LUT and briefly discuss the neurotransmitters involved and known details related to species/sex differences and developmental changes. Section 3 provides a brief summary of efforts to model the various neural components of the LUT for the purpose of understanding how they might participate in control. We list challenges in modeling the control of LUT at cellular and network levels and finally provide a brief description of an on-going effort to develop a biophysical model of the system.

2. Neurobiology of the mammalian lower urinary tract

2.1. General anatomy of LUT

The urinary bladder is a sphere-like hollow muscle that collects and stores urine from the ureters until it is stimulated to contract and evacuate the urine through the urethra – a process termed as micturition. The bladder is composed of a smooth muscle chamber, denoted the body, and a neck or posterior urethra, which serves to funnel urine into the urethra. At the base of the neck, a smooth muscle sphincter, the internal sphincter (IUS), prevents outflow to the urethra until a certain pressure is achieved. The urethra is a tube by which urine exits the bladder and flows to the exterior of the body. At the other end of the urethra is the EUS, which is a skeletal muscle sphincter, and the only voluntary component within the LUT. The main mechanical functions of sphincter muscles are controlled constrictions and relaxations. EUS allows conscious control over micturition and can be closed against fluid pressure by top-down signals from PSC. The coordination between EUS and bladder is mediated by complex neural circuits at the brain, spinal cord and ganglionic levels, and is crucial for proper functioning of the LUT. From a control point of view, internal changes such as convulsions, dysfunctions and infections, and external factors such as coughing, sneezing, cold, and fear, impact functioning of the EUS [4][5].

2.2.Neural Anatomy of LUT

The neural anatomy of the LUT can be viewed as having three main pathways labeled sympathetic, parasympathetic and somatic pathways. Although the functions of these pathways are similar in most species, differences exist between rat, mouse, cats and humans. The sympathetic pathway is responsible for relaxation of the bladder and contraction of the IUS. the parasympathetic pathway does the opposite by relaxing the IUS and contracting the bladder. These bladder contractions can be periodic non-voiding or voiding contractions. The somatic pathway implements the conscious control of micturition via the EUS.

The sympathetic, parasympathetic and somatic pathways carry sensory information from LUT to the spinal cord (hypogastric nerve) and efferent signals from the spinal cord to LUT (pelvic and pudendal peripheral nerves). Primary sensory afferents of the LUT project to spinal interneurons and can initiate and modulate several spinal level reflexes. These afferents may be involved in reflex integration at local ganglionic levels, and/or project to higher order centers to convey bladder fullness sensation to facilitate voluntary voiding. Pressure, stress, or toxins can also activate these afferents [6]. Efferents control the functional output of bladder by timed contraction or relaxation of the bladder, IUS and EUS. Summary of the neural anatomy can be found in (Fig 1) below adapted from Inskip et al.[7]

** Figure (1) Biology figure landscape full page with summary

Sympathetic:

Ganglia. It is estimated that in each hypogastric nerve about 1,300 afferent neurons, about 1,700 preganglionic neurons, and about 17,000 postganglionic neurons project their axons [8]. In Rats, Hypogastric ganglion contains around 3078 ± 193 (mean ± SEM) neurons and are known to exhibit both tonic and phasic firing patterns (Table1)[9]. However, several of these neurons innervate different visceral organs. So, it is difficult to isolate and obtain data from the ones innervating only the LUT.

Hypogastric efferents. Sympathetic efferent innervation of LUT arise from preganglionic neurons located between thoraco-lumbar (T10-L2) spinal segments in humans and lumbar (L1-L2) spinal segments in rats which project to postganglionic neurons in inferior mesenteric ganglion (IMG) and pelvic plexus (Fig 1). Axons from these postganglionic neurons innervate the body and neck of the bladder, and the urethra. These sympathetic postganglionic nerves release norepinephrine (NE) and contribute to storage mechanisms by contracting IUS (mediated by α-AR expressed in trigone, bladder neck and urethra) and relax the detrusor muscle (mediated by β-AR). [7]

*Hypogastric afferents***.** Hypogastric afferents are sensitive to small changes in bladder pressure caused by intrinsic detrusor contractions, across a wide range of pressures and tension [10]. The afferent signals in the pelvic and hypogastric nerves qualitatively appear to carry similar information and no functional classification can be made based on just afferent activity; the difference seems to be primarily related to the neurons they interact with at spinal level. Most of these afferents to the rostral lumbar segments terminate in laminae I, V–VII, and X [11]. Urethral afferents in hypogastric nerve can be both myelinated and unmyelinated and respond to urine flow and urethral distention. These urethral afferents exhibit rapidly adapting responses [12]. Hypogastric afferents are often active with bladder empty [13] and transection or pharmacological blockade of the sympathetic nerves can reduce urethral outflow resistance, reduce bladder capacity, and increase the frequency and amplitude of bladder contractions

recorded under isovolumetric conditions, thus reinforcing their functional significance during storage mode [14]. Hypogastric nerve also contains nociceptive afferents which are activated by chemicals and irritants.

Parasympathetic:
Ganglia. In rats, the number of labelled neurons in MPG that connect to bladder are 715 ± 126 neurons in 6 ganglia [15]. However, these labelled neurons are seen in penile, hypogastric, pelvic and accessory nerves. These neurons lacked dendrites and have soma cross section area of 309±7 μm^2 and are known to exhibit both tonic and phasic firing patterns [16].

Pelvic efferents. Parasympathetic inputs to LUT originate at the sacral level in humans (S2-S4) and at the lumbosacral level in rats (L6-S1). These inputs synapse onto neurons in the major pelvic ganglion (MPG) at the post ganglionic level. Axons from MPG innervate the body and neck of the bladder and the urethra; they excite the bladder and inhibit the IUS. Inhibition of IUS is mediated by nitric oxide (NO) (Fig 1). Parasympathetic excitatory transmission is mediated by ACh acting on muscarinic receptors (mAChR) especially the M3 subtype; when activated triggers extracellular Ca^{+2} release and results in contraction of detrusor smooth muscle (DSM). In some animals a noncholinergic contraction also occurs that is resistant to muscarinic receptor blocking agents. Adenosine triphosphate (ATP) is the excitatory transmitter mediating the noncholinergic contractions. Excitation of the bladder smooth muscle via ATP is also termed purinergic transmission [17], occurs via the action of ATP on P2X receptors (P2X1 in humans and rats) which are ligand gated ion channels. Purinergic transmission has an important excitatory role in animal bladders but is not important in the normal human bladder, except in patients with pathological conditions [18].

*Pelvic afferents***.** Pelvic afferents have cell-bodies in the dorsal root ganglion (DRG) and innervate the bladder as well as urethra and transmit afferent signals to the spinal cord at the lumbosacral level, into laminae V–VII and X at the base of the dorsal horn. They are broadly classified into mechanosensitive Aδ and C-fibers (Fig 1). The pelvic nerve carries "pressure-related" sensory information from the bladder to the spinal centers. Aδ mechanoreceptor afferents are myelinated and respond to both passive distention and active bladder contractions. Unmyelinated C-fibers that are not mechanosensitive respond to chemical irritants such as capsaicin and turpentine oil or cold. C-fibers are not active during normal physiological conditions of continence or micturition but are selectively active at high bladder pressures [10]. Some C-fibers are mechanosensitive and are less excitable than Aδ fibers. Some small number of pelvic afferents (both Aδ and C) track bladder pressure in exclusively high-pressure conditions and are quiescent at low pressures. This is most likely due to hyperdistention of DSM and the information conveyed is a sensation of pain.

Aδ mechanoreceptor afferents have conduction velocities in the range of (2.5 -15 m/s) [19] and are typically silent when the bladder is empty but display a graded increase in firing rate during slow filling, at pressures below 25 mmHg [20]. Basal firing rate of pelvic afferent neurons is ~1Hz when bladder volume is low [21] ; the maximal firing rates range from 15 to 30 Hz in cats. Four types of mechanosensitive fibers were identified by stretch, stroke and probe – Muscle (63%), muscle-urothelial (14%), serosal (14%) and urothelial (9%). Pelvic afferents can also be classified, based on pressure thresholds, into low threshold (LT) (65%-80%) and high threshold (HT) (20%-35%) categories [22]; for rats, these thresholds (mmHg; mean± std dev) are reported as 5.7 ± 1.0 for LT and 34.0 ± 2.5 for HT [23].

In awake behaving cats, low voiding efficiency and improper micturition are observed with chronic pelvic transection; no effect was observed with hypogastric nerve transection[24]. The normal micturition reflex is triggered by myelinated Aδ-fiber afferents [25, 26].The C-fiber afferent neurotoxin (capsaicin), does not block normal micturition reflexes in cats and rats, and are believed to not be essential for normal voiding [27]. Stimulation studies in rats revealed that C-fiber afferent neurons (24 μm diameter) have an inward current which is capsaicin sensitive and produce a TTX resistant spike with phasic firing and Aδ afferent neurons (33μm diameter) have an inward current which is insensitivity to capsaicin and is not TTX resistant and exhibit tonic firing [28].

Somatic:

Ganglia. There is no postganglionic stage in the somatic pathway. In the cat pre-ganglion, 45% of EUS motoneurons fired spontaneously with frequencies ranging from 12-27Hz [29]. Also, it has been demonstrated in the cat that during micturition, the urethral sphincter motoneurons hyperpolarize and hence slow down their firing or become quiescent [30].

Pudendal efferents. The only somatic-LUT connection is to the striated muscle of external urethral sphincter. Motor neurons in Onuf's nucleus located in the anterior horn of sacral S2-S4 segments in humans and lumbosacral L6-S1 segments in rats have long axons which directly innervate EUS via the pudendal nerve without any postganglionic targets in between. Their axons release ACh that act on nicotinic receptors (nAChR). (Fig 1)

During bladder filling pudendal motoneurons are activated by low level afferent input, whereas during micturition the motoneurons are inhibited by inhibitory input from supraspinal centers. This inhibition is dependent on supraspinal mechanisms and so is weak or absent in chronic spinal animals and in paraplegic patients. During filling, in spinal injured patients, the inhibition to motoneurons during micturition is lost and excitatory sphincter reflex pathway commonly initiates a striated sphincter contraction the same time as contraction of the bladder causing dyssynergia between bladder and sphincter, and interferes with bladder emptying; this phenomenon is termed detrusor-sphincter dyssynergia (DSD) [31].

*Pudendal afferents***.** Pudendal afferents have cell bodies in the dorsal root ganglia and enter the dorsal horn at the sacral or lumbosacral level (L6-S1 in rats, S1-S3 in cats, and S2-S4 in humans. Pudendal nerve afferent pathways of the cat, rat and monkey terminate in lateral laminae I, V–VII, and in lamina X [32, 33]. Pudendal afferents carry information related to pressure and flow rate from the urethra and coordinate urinary continence and micturition across many species.

Passing fluid through the urethra, irrespective of direction, causes pudendal nerve afferents activation at a much lower pressure compared to pelvic and hypogastric nerve afferents, which are activated by high-pressure flow that caused a distension of the urethra. Pudendal afferents are known to exhibit a slowly adapting tonic discharge that increases with bladder filling [34, 35]. Conduction velocities of pudendal nerve afferent fibers were found to communicate twice as fast (45 m/sec) as the pelvic nerve afferent fibers (20 m/sec) when responding to electrical stimulation of the urethra in cats [36].

At low bladder pressures, fluid entering the urethra can trigger an EUS contraction to maintain continence [37]. Studies in cats have also suggested that neurons in PSC provide a tonic excitatory input to the EUS motoneurons [38]. Electrical stimulation in PSC excites the EUS motoneurons and induces contractions of the EUS [39]. EUS also promotes continence via a feedback mechanism; contraction of the EUS induces firing in pudendal afferent fibers, which

activate spinal inhibitory interneurons involved in the micturition reflex to inhibit parasympathetic preganglionic neurons (PGN) to suppress reflex bladder activity [40]. In animal nerve transection experiments; by eliminating pudendal sensory feedback, voiding efficiency is reduced, suggesting that this sensory feedback critically interacts with the network that produces normal voiding behavior [41].

2.3. Neurotransmitters and receptors

Synaptic transmission in all ganglia (e.g., IMG, MPG) is mediated by ACh acting on different nicotinic receptors. However, there is neuromodulation at both pre and post synaptic receptor sites. Parasympathetic postganglionic neurons (MPG) release the excitatory neurotransmitter ACh / ATP, which contracts bladder smooth muscle, and nitric oxide (NO), which relaxes urethral smooth muscle [42,43]. Sympathetic postganglionic neurons (IMG) release NA (Noradrenaline) which relaxes DSM and contracts the urethral smooth muscle [44]. Somatic axons (motor neurons in Onuf's nucleus) release ACh and activate nicotinic cholinergic receptors on EUS that results in contraction.

Muscarinic receptors. Axons from MPG innervate DSM and release ACh which stimulates M3 muscarinic receptors that in turn triggers intracellular Ca+2 release that results in contraction of bladder. M3 receptors are the primary receptors in DSM, but M2 receptors are also expressed in large numbers, as much as 3-fold [45]. M2 receptors appear to play a functional role in 'recontracting' DSM after relaxation [46]. M2 receptors when activated can suppress the inhibitory mechanisms mediated by β3 receptors and contribute to bladder contractions [44]. In the normal bladder, muscarinic receptors are the primary effector of contractility in DSM, and these receptors are the main target for therapies aimed at symptoms of overactive bladder (OAB).

Purinergic receptors. Purinergic receptors respond to ATP, ADP, and adenosine. These receptors produce non-cholinergic contraction of DSM. The primary purinergic receptor present in DSM is P2X which are ligand-gated ion channels (Fig 1). Purinergic transmission doesn't seem to be playing an important role in normal human bladders but are important in animal bladders. However, purinergic signaling emerges as more prominent role in disease (Detrusor overactivity (DO), partial/chronic outlet obstruction and interstitial cystitis) and with aging [47]; potentially accounting for up to 65% of the total contraction force [44].

Adrenergic receptors (ARs). ARs expressed in the bladder mediate relaxation and the ARs expressed in urethral outlet mediate contraction in response to sympathetic innervation (Fig 1). The major receptor subtypes present in the human bladder are α1 and β3; however, β-receptors are expressed more compared to α-receptors [48]. The effects of α1-AR stimulation on smooth muscle contraction are rather weak, therefore, appear to play only a minor functional role in the detrusor, but play a prominent role near the bladder outlet in terms of maintaining outlet resistance [49]. The β3-receptor subtype is most expressed in human bladders and accounts for >95% of β-receptors. NE is released from the sympathetic efferents to activate β3-receptors and results in smooth muscle relaxation.

2.4.Differences in LUT circuit among species, sex and developmental changes

Species. Efferent innervation to LUT originates from different spinal sections among species: parasympathetic (pelvic nerves (S2-S4 in humans and L6-S1 in rats)), sympathetic (hypogastric nerves (T10-L2 in humans and L1-L2 in rats)), and somatic nerves (pudendal nerves (S2-S4 in humans and L6-S1 in rats)).

At the postganglionic level, morphology of the ganglion cells varies. In the rat MPG [50] and mouse IMG [51] the cells are (20-30 μm) in diameter and have no dendrites or only a few short dendrites. In cats, neurons in pelvic and bladder ganglia are larger (40–60 μm) and have a few dendrites. Variations in conduction velocities, patterns of convergence and characteristics of transmitter release have been observed under different physiological conditions [52]. Preganglionic parasympathetic input to the bladder ganglia are carried by myelinated (B-fiber) axons in cats [53], unmyelinated C-fibers in rats and mouse [51,54] and a mixture of B- and C-fibers in guinea pigs [55].

Considerable differences have been noted in synaptic transmission regarding the properties of the preganglionic input, and facilitatory and inhibitory synaptic mechanisms. In the rat, single presynaptic stimuli in the pelvic nerve elicit large amplitude EPSPs in postganglionic neurons [50,54].These responses do not change in amplitude during repetitive stimulation with frequencies (0.25-20 Hz). However, the discharges decrease in amplitude at high frequencies (30–50 Hz). So, in mouse, rat, and guinea pig, parasympathetic ganglia may function as relay and not integration centers [50,55]. In cats, single or low frequency stimuli (<0.25 Hz) elicit small amplitude EPSPs which gradually increase in amplitude during continuous stimulation at frequencies of (1-20 Hz) and maximal facilitation requires 15 to 25 stimuli in a train and persists for 30 to 60 s after termination of high frequency stimulation [56, 57]. This facilitation, also observed in rabbits, can suppress low frequency efferent activity and amplify high efferent activity, thus acting like a high pass filter.

Sex. Apart from the obvious anatomical differences among sexes in different species, there are considerable differences in physiology, morphology and function. Anatomically, DSM is thicker in men than women, as greater voiding pressure is needed to empty the bladder through the longer urethra of males [58]. Also, in male, the IUS is composed of smooth muscle that is arranged into long inner longitudinal and outer circular layers [59]. However, in women, the longitudinal cells do not form IUS, the bladder neck and proximal urethra form a functional rather than an anatomic internal sphincter. Contractility of DSM with cholinergic stimulation is found to be more sensitive in female rats than male [60]. Relaxation of bladder neck and urethra of male rabbits is more than in females with field stimulation [61] and gender doesn't seem to influence the contractility of the human bladder [62]. Regarding receptors, male rabbits have equivalent amounts of α1- and α2-ARs, whereas the females have a significantly denser population of α2-ARs [63] and cholinergic transmission was predominant in the male guinea pig bladder than the female bladder. And the opposite is true for purinergic transmission [64]. Differences also exist in voiding patterns between male and female rats. During voiding, maximum flow rate was lower and micturition time was shorter in female rats as compared to males. When we consider dysfunctions, it has been observed that overactive bladder (OAB) symptoms are more frequently observed and are significantly higher in women than in men but it is quite opposite in urinary urge incontinence (UUI) and DO symptoms [65 - 67].

Neonates vs adult. Voiding in neonates is dependent on pudendo-bladder reflex mechanism which is triggered when the mother licks the genital or perineal region of the young animal (e.g., rats and cats) [68, 69]. The pudendo-bladder reflex circuit is in the sacral spinal cord and has afferents coming in from the pudendal nerve and efferents transmitted in the pelvic nerve. In newborn kittens and rats, the pudendo-bladder reflex is essential for survival because urinary retention could be fatal if the newborn is away from the mother [70, 71]. Upon maturation, reorganization of synaptic connections in bladder reflex pathways happens which downregulate

neonatal spinal mechanisms and upregulates conscious voiding control via supraspinal mechanisms. Spinal cord injury in adult animals and humans which interrupts supraspinal-spinal cord connections causes the reemergence of the neonatal perineal/pudendal-to-bladder reflex. In neonates some supraspinal mechanisms may already be functioning to suppress the spinobulbospinal micturition reflex pathway allowing micturition to be controlled by primitive spinal reflex mechanisms [72].

3. Physiology of neural control of LUT & Computational Modeling

3.1. Filling vs Voiding

During filling, distension of bladder produces low-level afferent firing. At this stage parasympathetic efferents are quiescent and sympathetic and somatic efferents are active. Intravesical pressure measurements in both humans and animals reveal low and relatively constant bladder pressures when bladder volume is below the threshold for inducing voiding (Vol_{DEC}). Also, during filling, EUS electromyogram (EMG) show increases in the activity of the sphincter reflecting an increase in efferent firing in the pudendal nerve and an increase in outlet resistance that contributes to storage of urine [2].

Voiding can be triggered either voluntarily or involuntarily. In human infants, as the bladder fills, bladder afferent signals increase in strength until they exceed a certain threshold in the brainstem, specifically the periaqueductal gray (PAG). In the absence of any controlling influences, the pontine micturition center (PMC) is activated, the urethral sphincter relaxes, the bladder contracts, and voiding occurs [73]. This mechanism constitutes the involuntary voiding. In adults, increased afferent firing from inline tension receptors in the bladder produces firing in the parasympathetic pathways, inhibits sympathetic pathways and somatic pathways. This phase consists of an initial relaxation of the urethral sphincter followed by a contraction of the bladder, an increase in bladder pressure, and the flow of urine. The generation of conscious bladder sensations and the mechanisms that underlie the switch from storage to voluntary voiding involve cerebral circuits above the PAG [2].

Stimulation based experimental data. Several stimulation experiments proposed solutions to different LUT dysfunctions. Here we discuss one such stimulation - Pudendal nerve (PN) stimulation which has been replicated in a computational model [74]. PN stimulation has been proposed as a method to restore continence and micturition. This is possible because pudendal afferent stimulation can differentially activate spinal circuits responsible for reflex inhibition or excitation of bladder [75,76]. Low frequencies for PN stimulation (2-15 Hz) inhibit bladder contractions and increase bladder capacity and thus helps with continence. GABAergic mechanism in lumbo-sacral spinal column is evoked by this low PN stimulation and inhibits the excitatory control of the bladder, rather than activation of a bladder relaxation pathway [77]. High frequency PN stimulation (25-50 Hz) evoked contractions in bladder but 33 Hz pudendal afferent stimulation caused sustained SEC (stimulation evoked contraction) for bladder volumes >70% Vol_{DEC} (Volume threshold for distension evoked contractions) whereas 10 Hz caused SEI (stimulation evoked inhibition) [78, 79]. Pudendal afferent stimulation at 33 Hz failed to evoke robust bladder contractions (mean bladder pressure >10 cm-H_2O) below ~70 % of the volume necessary to evoke DECs showing volume dependence [80].

3.2. Brief history of modeling the LUT & challenges

The earliest reported computational model of the LUT was of a dog bladder. The authors assumed the bladder to be spherical with a structure that collapsed with increasing pressure. They used recordings from stimulations of female dog bladders to determine their feedback system with state space methods dependent on their measured motor neuron firing rates [81]. This general approach was followed throughout most of the later modeling attempts. Another group, Bastiaanssen et al. [82] developed a biomechanical model of LUT based on urine outflow data from patients, but further simplified their neural component to disregard firing rates, directly converting pressures to muscular contraction via empirically determined state variables. One of the earlier papers to attempt to model neural computation used a state-space formulation for each known neural ganglion. This group added levels of neural components incrementally and varied excitatory and inhibitory connectivity [83,84].

Since 2010, there has been a resurgence in modeling the neural circuit of micturition more realistically. Much of this has been devoted to determining the interactions between the supraspinal micturition centers (PMC/PAG, cortex, etc.) and the spinal ganglia or the interaction between the autonomic and somatic systems at the spinal level. De Groat's group has focused on the former, while Grill's group focused on the latter. De Groat's group created a model with simple monosynaptic weighed integration of binary neurons in the spinal cord [3]. Their supraspinal neurons were based on recordings taken from the PMC and PAG, as their overall goal was to understand the role of the supraspinal switching circuitry in micturition. Grill et al. developed an integrate-and-fire model of the spinal circuit at lumbosacral level of cat [74]focusing on the afferent pudendal stimulation. They have the most detailed model of connectivity at the spinal level; however, they ignore the sympathetic nervous system in their model. Their results are centered on studying the spinal circuitry involved in pudendo-vesical reflex.

Challenges to modeling cellular network neurophysiology.

The role of computational modeling is to study complex functions with relatively simple principles while preserving the necessary realism in the model which can shed light on the inner working at different levels of organization. However, when trying to model a functional circuit such as the LUT, with gaps in data, several assumptions need to be made using informed judgement.

Ganglion level. There is much overlap in the neurons which project to visceral organs and it is hard to know LUT specific neurons because all the axons are bundled together in the efferent nerves. So, several aspects required to model such as interconnectivity if any, synaptic divergence and convergence are unknown. The functional role of most postganglionic neurons is predominantly spatial amplification and filtering of preganglionic inputs. It has been suggested that after spinal transection the firing patterns of these neurons can change (Ex: from tonic to Phasic). These postganglionic neurons are subject to modulation from several neuromodulators at both pre and post synaptic sites. However, the implications of these changes are yet to be studied and models provide a great framework in that regard.

Cellular level. To model a biophysically constrained neuron, we match electrophysiological data such as passive properties (Resting membrane potential (V_{Rest}), input resistance (R_{IN}) and time constant (τ)) and active properties such as (firing patterns and FIR curve). To reproduce these electrophysiological characteristics, experimental data related to all the properties are typically obtained from intracellular current-clamp studies, if the channel types present in the neuron are

known. These neurons come with markedly different morphology across species, some with no dendrites and other with few. In order to capture the necessary essentials, we need answers to questions such as: Can the properties of the neuron be explained by a single compartment or do we need more compartments? Are the location of synapses important? For a systematic review of the methodology to build single cell models please refer to Guntu et al. [85].

To model the LUT circuit a challenge was that single cell data had to be compiled and integrated across different species and even from neonates and adults. It is noted that this is typical in computational studies involving brain regions in general, with the assumption being that characteristics of channels and other neuronal properties are similar across brain regions. For instance, in the case of LUT, there are relatively few studies that thoroughly characterize the firing patterns innervating efferent neurons, with the primary focus of the literature being on neurons of the MPG (for example see references [16,86-88]). There are even fewer studies that specifically characterize ionic conductances underlying those firing patterns. Finally, distinct cell types have been reported in the MPG, based on their outputs – at a minimum those that fire tonically as a result of depolarization, and those that show phasic output [86]. The existing literature on underlying membrane conductances is further limited by those that discriminate between these cell types in their analysis. Only a subset of membrane conductances of MPG neurons have been studied in voltage clamp, and many of these represent mixed currents. For example, sodium currents have only been measured in one study [89], and this same study separated at least three K+ current subtypes in dissociated cultured neurons [89]. Calcium channels have been fairly well documented, including voltage-clamp studies of both N- and T-type currents [90-92]. Finally, ATP-sensitive K+ conductance [93] and calcium-sensitive chloride currents [94] have been partially characterized. However, these studies have only begun to provide a comprehensive characterization of membrane currents of the pelvic ganglion neurons, and represent a diverse set of model organisms, pharmacological isolation, and cell-type specific localization.

Synaptic level. Synapses at all levels can be classified as excitatory and inhibitory and there is evidence of facilitating and depressing synapses as well. Neurotransmitters and receptors involved in the LUT circuit are highlighted above in section 2. Transmission through efferent nerves is either cholinergic or purinergic. A majority of transmission at postganglionic level is cholinergic and this transmission can be modulated at pre and postganglionic sites. Neuromodulation effects may be substantial on the computational properties of a neuron, but concrete experimental data are not yet available to incorporate these effects into a model.

In addition to membrane conductances and properties, even fewer studies have characterized synaptic currents. It is known that the cholinergic synapses of the MPG are largely carried by α3β4 subunits [95], and some of the integrative properties of these synapses have been studied [88,96]. However, there is relatively little information about the full spectrum of synaptic properties in the ganglia of the LUT.

Network level. The afferent fibers consist of high-threshold and low-threshold types and functioning of LUT is state dependent i.e., depending on the state, several reflexes emerge and implement different functions in the LUT. Some reflexes are represented as mono synaptic while there is some evidence that they may be multisynaptic. Connectivity within ganglia is mostly unknown except we know that there is considerable divergence from preganglionic to postganglionic neurons e.g., PGN-MPG. While this brain stem switch itself is a complicated circuit, researchers have assumed the supraspinal input as a simple on-off switch. . While

extracellular measures of LUT network activity have been made in conjunction with bladder output (for example see [26,97]), a comprehensive recording of simultaneous and coordinated efferent and afferent network activity during filling and voiding of the bladder has not yet been well reported.

Interface with bladder. The purpose of the neural circuit described so far is to control the urinary bladder. Specifically, to determine how efferents arising from the model result in bladder contraction or relaxation and how bladder distention results in increased bladder afferent firing rate. Bladder stretch releases ATP and ACh which acts on P2X receptors or muscarinic receptors (M3) on the afferent nerve terminal as described above in section 2. The physiological details and complex interactions involved with bladder smooth muscles, urothelium, interstitial cells and afferent nerves is beyond the scope of this chapter and are reviewed in a hypothetical model of uroepithelial sensory web [98]. There have also been excellent efforts to model urinary bladder smooth muscle in the mouse [99]. The next logical step would be to incorporate the current model with bladder smooth muscle model [99] or even the mechanistic model of bladder [82]. To complete the picture incorporate model of supraspinal control by the higher order centers will need to be incorporated [3].

3.3. Preliminary biophysical model of the neural LUT.

We are currently developing and testing a biophysical model of the neural circuit of the LUT. The model contains EUS afferents, supraspinal PAG/PMC input and initial input for bladder afferents. At spinal level we modeled parasympathetic preganglionic neurons (PGN), sympathetic preganglionic neurons (Hypo), medial interneurons (IN_{M+}(excitatory) and IN_{M-} (inhibitory)), dorsal interneuron (IN_D), and Feedback interneuron (FB). At postganglionic level we have models for MPG and IMG neurons. We also modeled the somatic motorneuron (EUS_{MN}). All neurons are modeled using the Hodgkin-Huxley formulation with single compartments [100]. Single cells are matched to reproduce available electrophysiological data (Table 1). A consolidated figure of the LUT model is provided in (Fig 2) below.

**Figure (2) Model figure with summary (Landscape)

There are 10 cells in each location and the connectivity between groups of cells is implemented using a Gaussian distribution as with our previous models [109-111] There is no interconnectivity between neurons within a ganglion. All synapses are modeled as double exponential functions with rise and decay times. Our spinal interneuron network uses a depressing synapse with the EUS afferent and an assumed excitatory synapse with the PAG/PMC input to model IN_{M+} as a low-pass filter for the EUS afferent. An excitatory synapse with the EUS afferent was used to model IN_{M-} as a high-pass filter for the EUS afferent. The bladder is filled at a constant rate. We use tuning curves from McGee et al., [74] to determine bladder pressure from PGN firing rate and we use this calculated pressure to then estimate bladder afferent firing rate. Once developed, a reliable model for bladder dynamics can replace this 'tuning curve' approximation that we use presently. . The model is implemented in NEURON [112] and uses the BMTK library to implement functions [113]. The feedback network uses the PGN firing rate to calculate bladder pressure, and then the bladder pressure and bladder volume to calculate bladder afferent firing rate at user-specified intervals during the simulation. This implementation permits implementation of closed-loop feedback given initial trends of bladder afferent firing rate.

Current model reproduces the observed trends of firing rates during a typical fill and void cycle with firing rates that match experimental observations (Fig 3). Further details related to the model can be found in Guntu et al. [114]

**Figure (3) Model output for LUT circuit with 10 cells at each location.

A key with such modeling efforts is to match preliminary trends with simple structures, e.g., 1 or 10 cells in each ganglion. Further refinements can be pursued once the model is able to reproduce key experimental findings. Challenges to improving the model are as follows: Biological data related to single cell characteristics are scarce and from different species. Once such data are available, the single cell models can be improved. The present model includes only tonic cells and so phasic and other cells types that may be discovered need to be incorporated. Both intra- and inter- connectivity are not known for the various ganglia, and so different parametric cases can be explored, ensuring all the while that firing rates are within the biological ranges. With increasing fidelity, such models can also help explore the various known 'reflexes' during both normal and abnormal (e.g., spinal cord injury) functioning of LUT networks. An important use of such models will be to provide or falsify testable hypotheses for understanding functioning as well as for the design of interventions such as electrical stimulation.

Summary

This chapter focused on reviewing relevant literature with to the goal of highlighting the challenges in computational modeling of the neural control of the mammalian LUT. A brief overview of the anatomy, neurophysiology and neurotransmitters preceded the main focus on the neural components of the LUT. Differences among species, sex and developmental changes are also outlined to show the complexity in the underlying biology. To put the review in context, we provide a brief summary of ongoing modeling efforts related to the LUT circuit with 10-cells at each location. This preliminary model successfully simulated the firing pattern trends observed in experiments during the filling and voiding cycle. Lack of experimental data necessitates thoughtful assumptions that are informed by an understanding of the neural fundamentals. As cited, an important use of such models will be to generate testable hypotheses for understanding normal and pathological functioning of the LUT, and to provide guidance for the design of interventions, e.g., electrical stimulation.

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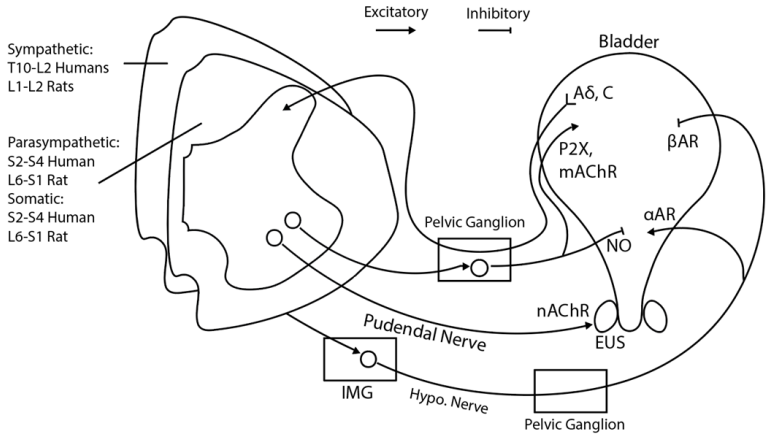
Figures:

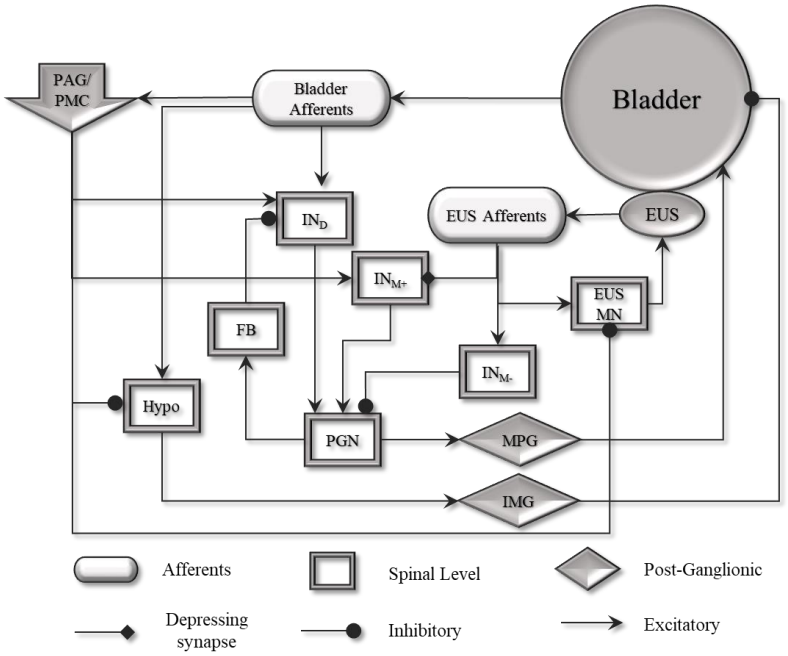
Innervation of the lower urinary tract (LUT)



- The LUT is comprised of the bladder, urethral sphincter and urethra.
- The LUT receives the bulk of its innervation from three nerves.
- The hypogastric nerve carries sympathetic innervation to the LUT; contributing spinal nerves exit the spinal cord (SC) between L1 and L2. Muscle activity for storage is mediated by α-AR expressed in the trigone, bladder neck and urethra (excitatory), and by β-AR expressed in the bladder dome (inhibitory).
- The pelvic nerve contains parasympathetic input originating in the sacral cord (L6–S1 in rat) and controls micturition via cholinergic muscarinic receptors (mAChR) expressed throughout the LUT.
- The human pudendal nerve exits the sacral SC and provides somatic innervation to the striated muscles of the external urethral sphincter; in rats, the pudendal nerve originates in the L6–S1 cord.
- In addition to their efferent function, each of these nerves carries afferent input from the LUT. Information about bladder distension is carried by mechanosensitive afferents (Aδ, C) found primarily in the pelvic nerve. These afferents signal the coordinated switch between storage and micturition. The pudendal and hypogastric nerves mostly contain nociceptive afferents, which are not depicted here.
- **Abbreviations**: AR, adrenergic receptors; DR g, dorsal root ganglion; EUS, external urinary sphincter; g, ganglion; IM g, inferior mesenteric ganglion; L, lumbar spinal cord; mAChR, muscarinic cholinergic receptors; n, nerve; nAChR, nicotinic cholinergic receptors; NO, nitric oxide; P2X, purinergic receptor; S, sacral spinal cord; (+) denotes excitatory synapses; (-) denotes inhibitory synapses.

Figure1: Consolidated biological figure of LUT innervation

Computational model of LUT



- All neurons specified are single cell Hodgkin-Huxley type matching available electrophysiology data.
- Synapses are modeled as double-exponential type with rise and decay time.
- Our spinal interneuron network uses a depressing synapse with the EUS afferent and an assumed excitatory synapse with the PAG afferent to model IN_{M+} as a low-pass filter for the EUS afferent.
- An excitatory synapse with the EUS afferent was used to model IN_{M-} as a high-pass filter for the EUS afferent, baseline bladder afferents.
- Generated inputs to the model include higher order PAG/PMC, EUS afferents and baseline bladder afferents.
- Our feedback network uses BMTK's simulation modification capabilities to collect the PGN firing rate, use it to calculate bladder pressure, and use calculated bladder pressure and simulated bladder volume to calculate bladder afferent firing rate at user-specified intervals during the simulation. This implementation allows for a closed-loop feedback given initial trends of bladder afferent firing rate.
- The calculations of bladder pressure and bladder afferent firing rate are currently based on tuning curves obtained from biological data.
- **Abbreviations**: MPG: major pelvic ganglion; IMG: inferior mesenteric ganglion; EUS: External urethral sphincter; IN_{M+}: excitatory medial interneuron; IN_{M+}: excitatory medial interneuron; IN_D: dorsal interneuron; PGN: parasympathetic preganglionic neuron; FB: feedback interneuron; Hypo: sympathetic preganglionic neuron. PAG/PMC: periaqueductal gray/ pontine micturition center denoting higher order input.

Figure2: Consolidated figure of LUT model

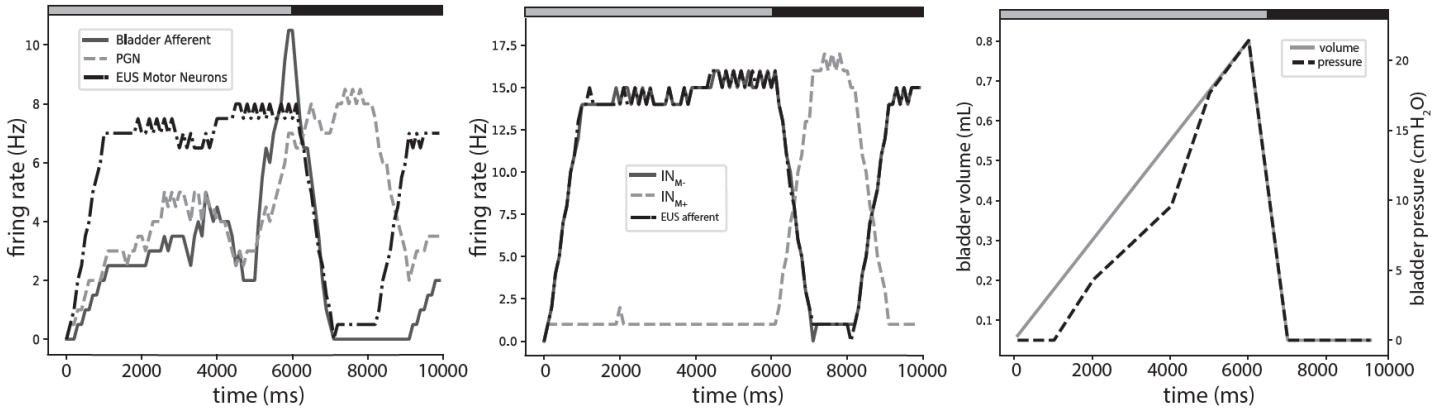


Figure 3: Filling vs Voiding in current biophysical model

(A) Firing rates of bladder afferents, preganglionic neurons (PGN) and EUs motor neurons; (B) Firing rates of medial interneurons (IN_{M+}, IN_{M-}) and EUS afferents; (C) Bladder volume and pressure profiles

Tables:

Table 1: Electrophysiological properties of neurons in LUT circuit (*: Rat, ↗:Mice, Δ: Cat)

Properties/ Neurons	V_{Rest} (mV)	Capacitance (pF)	R_{IN} (MΩ)	τ (msec)	Firing rate (Hz)	No. of Neurons
Bladder afferents [23][101]	-	-	-	-	0.06-40.4	55±6/ganglion
EUS afferents [102]	-	-	-	-	1-65	-
*IN_D [103]	-53.6 ± 2.54	-	822 ± 151	41.81 ± 5.4	21.83 ± 1.5	-
*$IN_{M+/-}$ [104]	-60 ± 1	94.8 ± 7.9	270 ± 25	22 ± 1.5	-	-
FB	-	-	-	-	-	-
*PGN [105]	-51.0 ± 1.1	26.5 ± 1.5	771.3 ± 70.7	-	-	-
↗HYPO [106]	-59.8 ± 7.4	32.8 ± 14.1	1140 ± 0.6	92.4 ± 43.7	-	-
↗MPG_T [16] [101]	-46.1 ± 1.64	28.9 ± 2.0	143.7 ± 14.0	4.0 ± 0.3	-	715 ± 126
↗MPG_P [16] [101]	-47.4 ± 1.3	28.7 ± 2.3	157.2± 14.5	4.6 ± 0.5	-	715 ± 126
ΔIMG [9] [107]	-53.7 ± 2.45	-	83.0 ± 13.1	5.8 ± 1.4	-	3078 ± 193
*EUS_{MN} [29]	-55.0 ± 8.0	-	11.1 ± 4.6	4.9 ± 1.5	-	-
PAG/PMC [108]	-	-	-	-	0-15	-