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Sengupta, Nilapratim; Brain, Keith; Manchanda, Rohit

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Mimicking Spontaneous Transient Depolarization in the Urinary Bladder Syncytium

Nilapratim Sengupta, Keith L. Brain, Rohit Manchanda

Abstract

The urinary bladder wall is composed of the detrusor smooth muscle (DSM) in which adjacent cells are electrically coupled to form a three dimensional syncytium. The structural complexity of the tissue is further enhanced by a distributed innervation pattern. Experimental techniques employed in order to analyze detrusor excitability have been unable as yet to provide a satisfactory understanding of either the normal electrical functioning of the tissue, or of the changes that come about in pathological conditions. A computational model of the bladder can serve as a virtual laboratory to gain deeper insight into the functioning of the bladder, both in normal and overactive states. Our work aims at exploring the interplay between factors that determines the spread of junction potentials in the tissue which is critical for generation of action potential and subsequent contraction of the bladder wall. Analysis of simulation results suggests strong correlation between structural complexity and functional aspect of the detrusor as well as point towards mechanisms underlying pathological scenarios.

Introduction

The urinary bladder is a visceral organ that stores and expels urine to the exterior. This is crucial for control of water and electrolyte balance in the body. Loss of control over urinary bladder function is prevalent among aging individuals as well as spinal cord injury patients. Besides pathophysiological implications, this significantly impairs the quality of life of an individual. In order to inspect the underlying cause(s) of bladder instability, one needs to understand its normal functioning. A comprehensive understanding of bladder biophysics is still lacking owing to technical challenges concerned with electrophysiological study of smooth muscle cells (*Manchanda, 1995*) which form the bladder wall. This smooth muscle tissue, called the detrusor, is the contractile component that helps in expulsion of urine and is thus of prime importance for bladder activity.

Electrical activity, observed in detrusor smooth muscle (DSM) cells of the urinary bladder, is quite varied and atypical. Specific reasons for such diversity in electrical activity are yet to be elucidated. However, understanding the basic structural layout and the sequence of events might help one hypothesize the probable causes. The innervation pattern in the tissue is of the distributed type where single nerve fiber may innervate multiple muscle cells, while a single muscle cell may receive neurotransmission from multiple nerve fibers, thus forming a “ground plexus”. Connections between terminal nerve fibers and individual cells are established at varicosities. In an innervated DSM cell, neurotransmitter release results in a transmembrane conductance change owing to opening of receptor operated ion channels. This leads to depolarization, which if supra-threshold, leads to the generation of an action potential. Moreover, owing to the syncytial nature of DSM, such signals spread across neighboring cells, the coupling being primarily effected by gap junctions. The spatio-temporal summation of generated potentials, in such a scenario, may be modulated by different aspects – be it transmitter release dynamics, density of innervation, magnitude and/or kinetics of conductance change (channel opening) or even distribution of gap junctions. A study of the interplay of these factors could be a starting step towards understanding the functioning of a normal healthy bladder. That will provide leads to causes for pathology, like bladder overactivity, and possibly direct the approach to their treatment.

In the scenario where experimental limitations restrict our understanding of bladder electrophysiology, a computational model, that has faithful correspondence with the physical system, can be subjected to investigations in an attempt to understand the bladder mechanisms in normal and overactive states. To investigate a complex system as this, one needs to build a simple model to begin with, then subsequently incorporate additional complexity. While it is the action potential that triggers contraction in a smooth muscle cell, the fundamental cellular signal is a passive (sub-threshold) junction potential that is elicited following release of neurotransmitter. Spatio-temporal integration of such junction potentials, when a threshold is exceeded, brings about an action potential. These junction potentials can be of two types: a. Excitatory Junction Potential (EJP); b. Spontaneous Transient Depolarization (STD). While an EJP is observed when a nerve action potential causes synchronous

neurotransmitter release across the ground plexus, an STD occurs owing to random asynchronous release of neurotransmitter across different varicosities or due to processes intrinsic within the smooth muscle cells; only the former will be specifically considered here. In this paper we report the results pertaining to spread of passive potentials (STDs) in a detrusor syncytium following stochastic neurotransmitter release and discuss them in the context of functional aspects of the bladder in normal and overactive states.

Methods

Computational modeling has been performed in NEURON, a simulation environment for neurons and neuronal networks. Given that smooth muscle syncytia have similar electrical properties to those of neurons, basic underlying electrical concepts, such as cable theory and excitable nature, will also apply in such electrical syncytia. Therefore, we will attempt to utilize the same platform for modeling the detrusor, the smooth muscle layer of the bladder wall.

A. Passive Detrusor Syncytium

The tissue model of the detrusor comprises of a cubical cellular network (*Appukuttan et al., 2014*) where each muscle cell is coupled to six neighboring cells via resistive shunts depicting gap junctions. It must be mentioned here that the focus of the study being the spread of passive potentials in the tissue, active ion channels were not incorporated at this stage. Parameters for individual cell are tabulated below.

Table 1: Parameters for DSM cell

Parameter	Value
Cell Length	200 μm
Cell Diameter	6 μm
Intracellular Resistivity	181 $\Omega\cdot\text{cm}$
Membrane Resistivity	138 $\text{k}\Omega\cdot\text{cm}^2$
Membrane Capacitance	1 $\mu\text{F}/\text{cm}^2$

Leakage Conductance	1/138000 S/ cm ²
Gap Junctional Resistance	33.33 MΩ

B. Neuromuscular Junction

The primary work at hand, while modeling electrical response of a muscle cell following neurotransmission, is to model the kinetics of conductance change at the autonomic neuromuscular junction (NMJ), where a nerve terminal establishes connection with a smooth muscle cell. The electrical equivalent for a single cell, in its passive state, is a parallel resistor (passive ion channels) and capacitor (lipid bilayer) network connected between the two conducting media – the intracellular and the extracellular fluids (ICF and ECF respectively). NMJ, in effect, can be incorporated in the cellular model as a gated parallel low resistance shunt with its own characteristic kinetics.

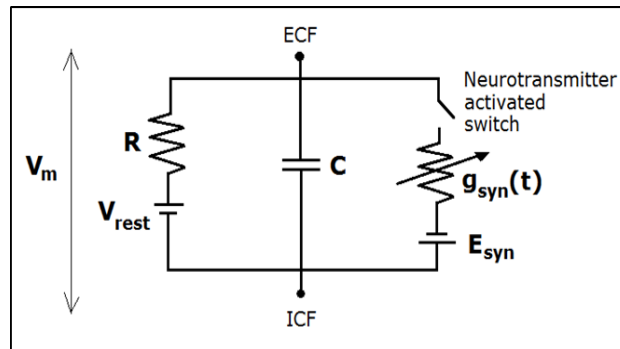


Figure 1: Electrical equivalent circuit for a cell with NMJ

In Figure 1 R , C and V_{rest} represent the net membrane resistance, capacitance and membrane potential at rest. Upon activation by neurotransmitter, the switch closes and time-varying conductance $g_{syn}(t)$ is expressed. E_{syn} is the synaptic or junctional equilibrium potential towards which the membrane potential is driven following transmitter release. The synaptic or junction current is given by:

$$i_{syn}(t) = g_{syn}(t) * (V_m - E_{syn}) \quad \dots(1)$$

The dynamics of conductance change reflects the kinetics of neurotransmitter binding and subsequent opening of receptor operated channels allowing a net influx of cations. In the physiological scenario, most junctional conductances have a finite rise time and their kinetics can be suitably modeled using an alpha function.

$$g_{syn}(t) = \overline{g_{syn}} * \frac{t-t_0}{t_{peak}} * e^{-(t-t_0-t_{peak})/t_{peak}} \quad (\text{when } 0 < \frac{t-t_0}{t_{peak}} < 10) \quad \dots(2)$$

$$= 0 \quad (\text{when } t < t_0)$$

Where,

$g_{syn}(t)$ = variation of conductance with time

$\overline{g_{syn}}$ = peak conductance

t_0 = onset time

t_{peak} = time to peak

C. Neurotransmission – An Event Based Approach

The kinetics of conductance change discussed above predict the dynamics of the post-junctional potential once the event of neurotransmission has happened. However, while modeling STDs, one must take into account the fact that the underlying phenomenon which gives rise to these spontaneous post-junctional potentials is the random release of neurotransmitters from varicosities distributed over the syncytium. The apparently independent nature of spontaneous neurotransmitter release at each release site suggests that such STDs are randomly distributed in time. Thus, in a given time frame, the count of spontaneous release should be governed by a Poisson process where the occurrence of an event in a given interval has no effect on the probability of a second occurrence of the event in that interval or any other. Now, if the number of occurrences of a random event in a given time frame be described by a Poisson distribution, the duration between successive events is provided by the negative exponential distribution. Thus for a randomly occurring event, such as

spontaneous release of neurotransmitter, the inter-event intervals (IEIs) follow a negative exponential trend.

In the model, the stochastic activation of each junctional conductance has been achieved using artificial spike trains where each spike corresponds to an 'event' of neurotransmitter release and such events are randomly distributed in time, IEIs being picked from a negative exponential distribution. The following figure illustrates this.

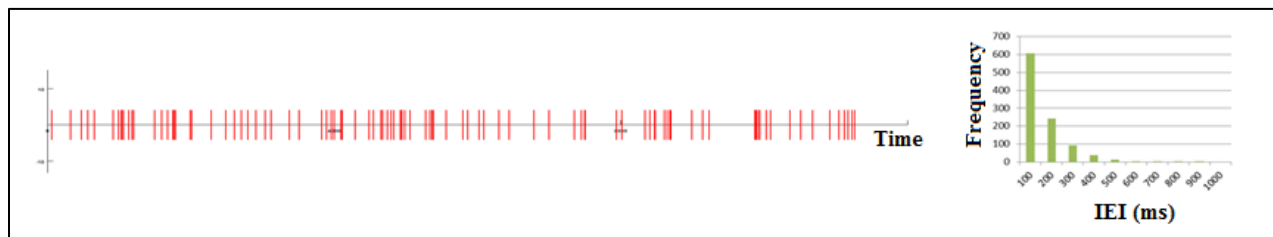


Figure 2: Raster plot depicting an artificial spike train and its IEI histogram

In case of our syncytial model, for mimicking STDs, not only do the spikes in a certain spike train need to be random, but each spike train, being delivered to individual NMJ, needs to be unique. Towards this end one may set different seeds and generate differing spike trains thus ensuring asynchrony across different varicosities.

Results

In the initial stage, a 21*21*21 (21-cube) cell model of the detrusor syncytium was endowed with NMJs (one per cell) and following random events of neurotransmission, the junction potential from a peripheral cell was recorded. Experimentally, intracellular recording (in the absence of nerve stimulation) of the membrane potential (V_m) from a single DSM cell (in a tissue preparation) presents STDs of various amplitudes spread out in time owing to spatio-temporal integration of propagated signals as well as those that are native to the recording cell.

Similar to electrophysiological recordings, simulation results produced STD patterns randomly spread out in time but superimposed on a basal depolarization (deviation of baseline potential

from the resting level) that suggested overactivity. Subsequent sets of studies revealed that the level of this basal depolarization correlated with both the density of innervation (percentage of cells endowed with a NMJ) as well as the mean inter-spike interval (ISI) of the artificial spike train that defines the neurotransmitter release instants for any given NMJ.

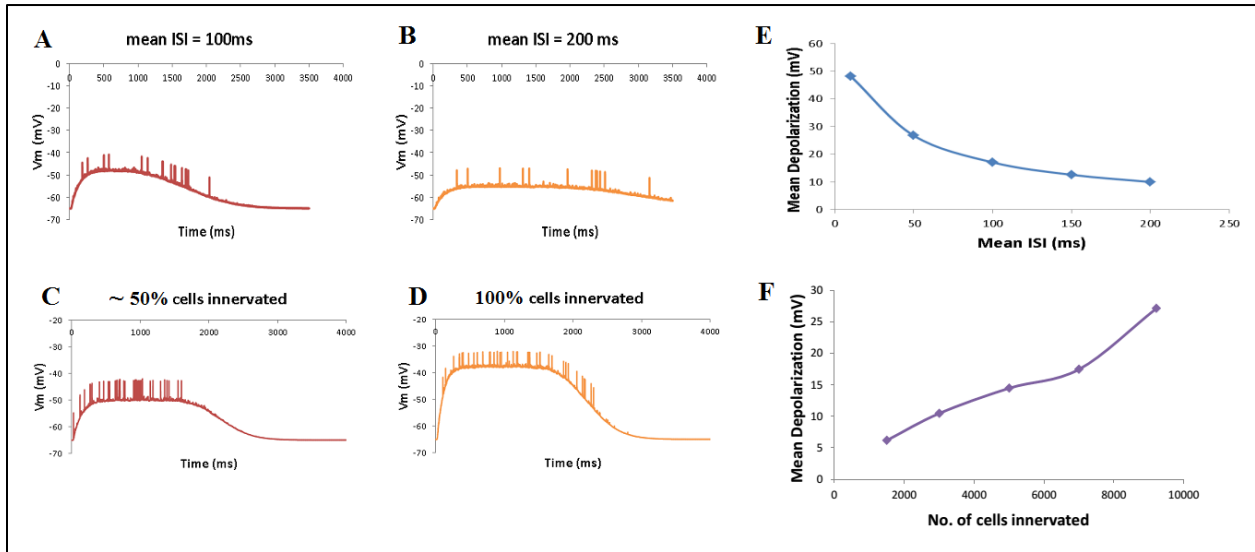


Figure 3: Dependence of basal depolarization on mean ISI and innervation density. A) V_m of peripheral cell with mean ISI = 100 ms, B) V_m of peripheral cell with mean ISI = 200 ms, C) V_m of peripheral cell when innervation is about 50%, D) V_m of peripheral cell when innervation is about 100%, E) Variation of basal depolarization with mean ISI & F) Variation of basal depolarization with degree of innervation

Figures 3A and 3B present the membrane potential (V_m) recording from the peripheral cell of a 21-cubed syncytium with 100% innervation density wherein the mean ISI of spike trains were 100 ms and 200 ms respectively. On the other hand, figures 3C and 3D represent membrane potential (V_m) recording from the peripheral cell of a 21-cubed syncytium with 50 ms as mean ISI for all spike trains wherein innervation densities were about 50% (5000 of 9261 cells) and 100% (all 9261 cells) respectively. Figure 3E depicts that the basal (mean) depolarization diminishes as the mean ISI is increased, clearly owing to lower probability of overlap between subsequent signals. Figure 3F demonstrates a positive correlation of basal depolarization with the density of innervation, which can be attributed to increased overlap of junction potentials when the rate of STDs increases.

When the composite effects of these two factors on basal depolarization were plotted on a contour plot, an interesting observation emerged.

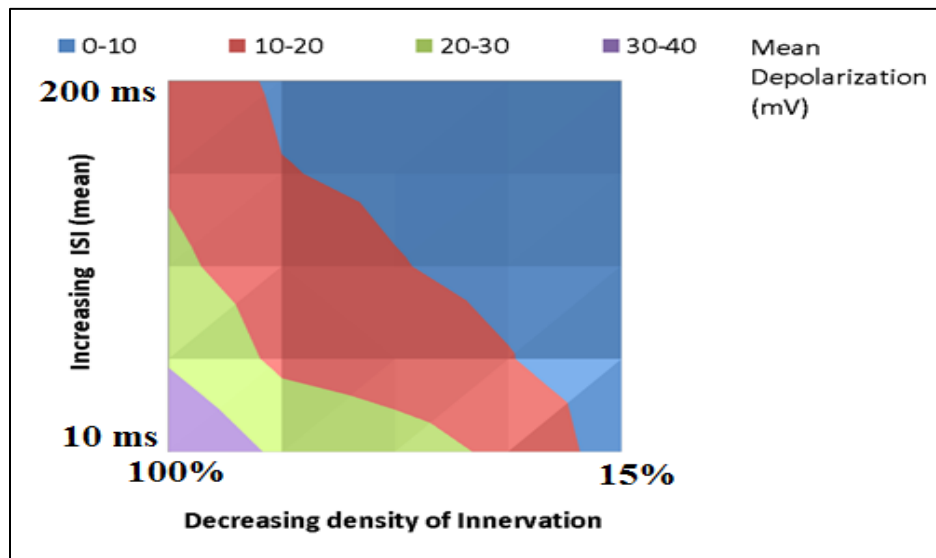


Figure 4: Contour plot demonstrating composite effect of mean ISI and innervation density on mean depolarization

From Figure 4 it can be observed that the level of depolarization is always low, irrespective of the mean ISI, when the innervation density is low. Hence one may infer that the effect of density of innervation dominates over that of mean ISI. This is corroborated by the fact that for densely innervated tissue, even with very high mean ISI, the mean depolarization is relatively higher than observed with sparsely innervated tissue.

These observations, together with analysis of electrophysiological recordings led us to perform a systematic variation of contributing parameters so as to obtain their optimized values in order to mimic the normal electrical activity of the detrusor syncytium. Satisfactory results were obtained with a 9-cubed syncytial network in which 5% of cells were endowed with NMJs, the mean ISI for each NMJ being of the order of 90 seconds.

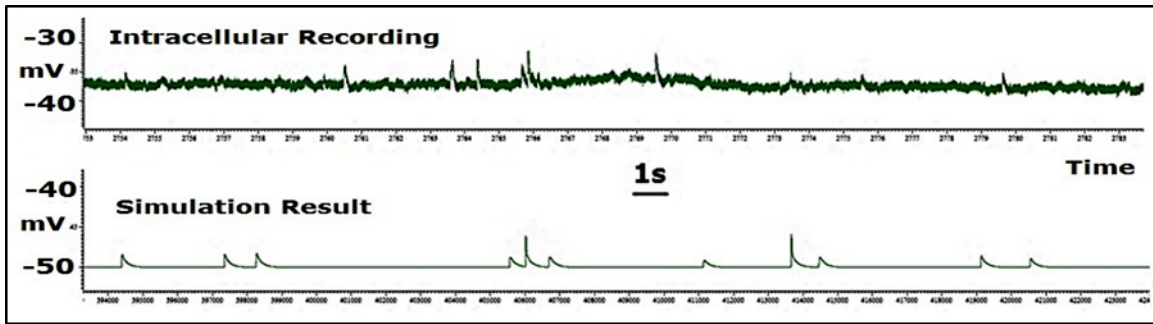


Figure 5: Comparison of electrophysiological recording and simulation result

Figure 5 depicts the simulation result from the computational model presented along with electrophysiological recording. Experimental recordings indicate that the mean interval between STDs falls in the range of 2 – 7 seconds.

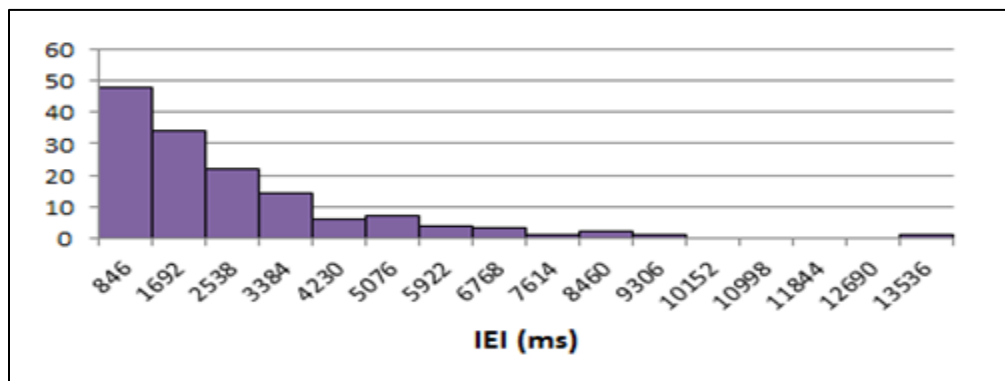


Figure 6: Inter event interval histogram of STDs (5% innervation density)

Figure 6 presents the inter event interval (IEI) histogram of STDs from simulated results which shows a negative exponential trend as one would expect for a stochastic process. The mean IEI was of the order of 3 seconds which is in accordance with electrophysiological data.

Discussion

The results from the model indicate that the spatio-temporal integration of passive potentials and their subsequent propagation is majorly affected by innervation density as well as the mean time between subsequent random releases at a NMJ. Previous reports suggest that DSM cells are organized into bundles where inter-bundle coupling is relatively poor as compared to intra-bundle coupling amidst neighbors (*Hashitani et al., 2001*). It is also reported that the

density of innervation in the detrusor is quite high. Such a physiological scenario is complementary to the model layout that has extensive coupling but sparse innervation. This suggests that it is the interplay of degree of coupling and innervation density that governs the spread of potentials in the detrusor, a well coupled and poorly innervated syncytium being able to mimic a poorly coupled and densely innervated tissue. It is a well-established principle that structure defines function. From that standpoint, it can be conjectured that in the detrusor, poor inter-bundle coupling allows localized contractions which are critical for imparting compliance when the bladder functions in storage mode. On the other hand, dense innervation might ensure synchronization of contractile activity through nervous input, thus allowing micturition.

The results presented also indicate that given a dense innervation, if the coupling between cells is enhanced (possibly through over expression of gap junctions), overactivity of the urinary bladder may result – as observed in terms of increased basal depolarization, making the tissue more excitable. This is in fact a proposed myogenic cause for detrusor instability (*Brading, 1997*). Furthermore, if the mean time between random spontaneous releases from varicosities get reduced, a heightened basal depolarization results, effectively making the tissue more excitable. This could be a neurogenic cause of bladder instability. An experimental method of testing this might be to add a toxin that decreases the ISI, such as alpha-latrotoxin, and measure subsequent change in the resting membrane potential and tissue excitability.

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