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Hayashi F[1], de Groat W[1], Roppolo J[1], Birder L[1], Griffiths D[1], Tai C[1], Bergamin L[1], Wu H[1], Kanai A[1] 1. University of Pittsburgh

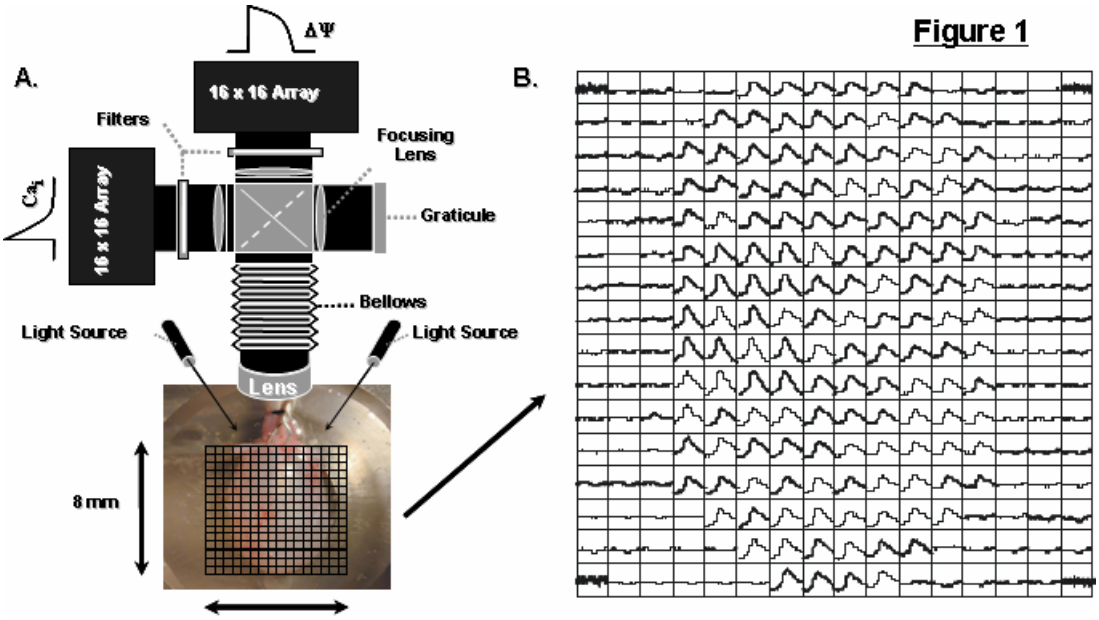
INCREASED GAP JUNCTION CONNECTIVITY AND FOCAL PACEMAKER ACTIVITY IN THE BLADDER FOLLOWING SPINAL CORD INJURY MAY LEAD TO URINARY INCONTINENCE - REVEALED THROUGH OPTICAL IMAGING

Hypothesis / aims of study

Detrusor overactivity in humans refers to involuntary contractions during the filling phase of the bladder, when the subject is not intending to void. These contractions may be either spontaneous or evoked and it has been suggested that they may require initiation by a pacemaker. This type of activity is also present normally in the bladders of neonatal rats, but is downregulated during postnatal development when neural mechanisms in the brain emerge as the dominant regulator of lower urinary tract function. However, non-voiding contractions re-emerge in adult rat bladders under abnormal circumstances such as spinal cord injury (SCI) and are believed to be analogous to detrusor overactivity in humans. There have been numerous attempts to characterize bladder pacemaker activity and the mechanism of detrusor overactivity using traditional methods such as cystometry, extracellular, intracellular and patch clamp recording techniques. However, it remains to be determined whether spontaneous excitation in the bladder originates from restricted specialized sites or occurs randomly, how excitation propagates within the smooth muscle and the mechanism underlying detrusor overactivity.

We hypothesize that spontaneous activity in neonates is initiated at a focal pacemaker site and propagates through the detrusor via gap junctions. In the adult, when neuronal innervation of the bladder is complete, this gap junction connectivity is downregulated. We further hypothesize that in spinal cord injury, when neural control is disrupted, gap junction connectivity is upregulated and coordinated spontaneous activity re-emerges. In order to test these hypotheses, we have developed optical imaging techniques to map action potentials and Ca^{2+} fluxes in the detrusor. The optical setup includes a dual camera photodiode array system that allows us to simultaneously record, at up to 4000 frames/s, action potentials, and intracellular Ca^{2+} transients from 256 sites in the bladder wall (Fig 1A). Accordingly, our aims were to apply this imaging methodology to investigate the origin(s) of spontaneous activity and the mechanism by which it propagates through the bladders of neonatal, adult, and spinal cord transected rats.

Figure 1



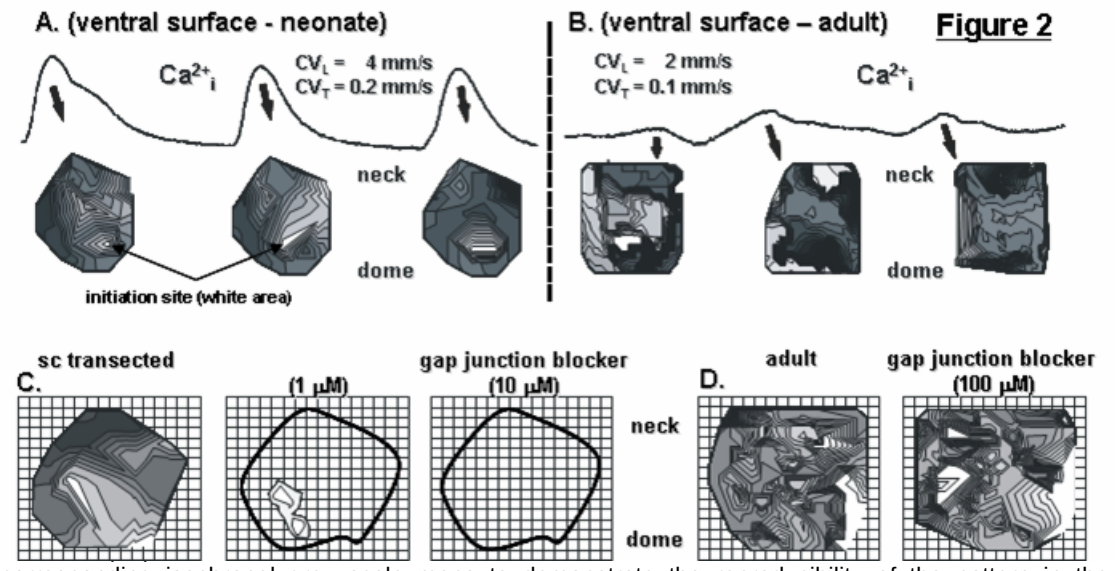
Study design, materials and methods

Neonatal (7-21 day-old), adult (4 mo-old) and spinal cord transected (T_8-T_9; 2 wks after injury) rats were anesthetized and their bladders excised, cannulated, and stained/loaded with voltage (di-4-ANEEPS) and Ca^{2+} (Rhod-2-AM) sensitive dyes. The bladders were placed in a 37°C bath, connected to a pressure transducer and imaged for spontaneous and electrically evoked optical signals from the ventral, dorsal or lateral surfaces. Isochronal maps (Fig 2A-D) were generated from the local activation

time-points for the 256 optical action potentials (using 1st derivative analysis) and intracellular Ca^{2+} transients (using cross-correlation analysis). An example of the raw intracellular Ca^{2+} data recorded from the bladder surface and used to generate an isochronal Ca^{2+} map is shown in Fig 2B.

Results

In neonatal bladders, a coordinated pacemaker activity originated near the dome resulting in smooth muscle Ca^{2+} action potentials (conduction velocity = 45 mm/s; not shown) that initiated intracellular Ca^{2+} transients (4 mm/s) which spread throughout the detrusor (Fig 2A). This resulted in large amplitude (15-25 cm H_2O) spontaneous contractions. In adult bladders, spontaneous activity originated at multiple sites (2B) and was of lower amplitude (2-5 cm H_2O). Following SCI, a pacemaker site re-emerged at the dome (2C), initiating large amplitude (15-25 cm H_2O) spontaneous activity similar to neonatal bladders. Simultaneous blockade of the effects of acetylcholine, ATP, substance P, nitric oxide and - and - adrenergic agonists failed to inhibit spontaneous activity in neonatal, adult, or SCI bladders. However, gap junction blockade (glycyrrhetinic acid, 10 M) reversibly abolished spontaneous activity in neonatal (not shown) and SCI (2C) bladders, but not adult (2D) bladders. Electrical stimulation of neonatal and SCI bladders after gap junction blockade evoked only a small area of depolarization. In contrast, stimulation before gap junction blockers activated a depolarization response that spread throughout the detrusor (not shown). All experiments were carried out in n≥6 bladders.



corresponding isochronal grey scale maps to demonstrate the reproducibility of the pattern in the neonate that was lost in the adult. In the grey scale maps, the white areas are the first and black areas are the last regions of the bladder to undergo a Ca^{2+} transient. In the neonatal bladder there is a repeatable initiation site (pacemaker) while in the adult bladder there are multiple initiation sites that change from one contraction to the next. This reproducibility re-emerges in the transected bladder (2C).

Concluding message

These findings suggest that gap junctions are necessary for focally initiated large amplitude spontaneous activity in neonatal bladders, and that this connectivity is lost during development and re-emerges following SCI. Spontaneous activity is coordinated, less chaotic and gap junction dependent in bladders from neonatal and SCI rats, but more chaotic and gap junction independent in bladders from adult rats.

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