Developing a functional urinary bladder: a neuronal context

Janet R. Keast*, Casey J. A. Smith-Anttila and Peregrine B. Osborne

Department of Anatomy and Neuroscience, University of Melbourne, Melbourne, VIC, Australia

The development of organs occurs in parallel with the formation of their nerve supply. The innervation of pelvic organs (lower urinary tract, hindgut, and sexual organs) is complex and we know remarkably little about the mechanisms that form these neural pathways. The goal of this short review is to use the urinary bladder as an example to stimulate interest in this question. The bladder requires a healthy mature nervous system to store urine and release it at behaviorally appropriate times. Understanding the mechanisms underlying the construction of these neural circuits is not only relevant to defining the basis of developmental problems but may also suggest strategies to restore connectivity and function following injury or disease in adults. The bladder nerve supply comprises multiple classes of sensory, and parasympathetic or sympathetic autonomic effector (motor) neurons. First, we define the developmental endpoint by describing this circuitry in adult rodents. Next we discuss the innervation of the developing bladder, identifying challenges posed by this area of research. Last we provide examples of genetically modified mice with bladder dysfunction and suggest potential neural contributors to this state.

Keywords: micturition, continence, pelvic ganglion, dorsal root ganglion, inferior hypogastric plexus, visceral afferent, neural development, organogenesis

Understanding the Endpoint: Innervation of the Adult Urinary Bladder

Overview of Bladder Innervation
The urinary bladder functions to collect and store urine so that it can be released at behaviorally appropriate times. These alternating phases of continence and micturition are precisely controlled by a hierarchical system of neural controls in the spinal cord and brain (Andersson and Wein, 2004; Beckel and Holstege, 2011; de Groat and Yoshimura, 2015). This circuitry connects with the bladder via a system of direct and indirect peripheral neural pathways summarized in Figure 1. In each component of this circuit, nerves involved in bladder control are intermingled with many other nerve types, as discussed in detail below.

This review will provide an overview of the features that govern the organization and function of bladder innervation in laboratory animals and humans. In general, the neural connectivity of the nerve supply to the bladder tissues is similar across species. However, it should be noted that while most contemporary studies use rodents, it was previously more common to use cats, which have larger nerves and are easier to study with neurophysiological techniques. Nevertheless, it is

Abbreviations: Ach, acetylcholine; DRG, dorsal root ganglion; NAd, noradrenaline (norepinephrine); NF200, neurofilament protein, 200 kd; TRPV1, transient receptor potential vanilloid receptor 1.
clear that rodents have limitations for studying some specific questions relevant to human clinical urology, such as urethral sphincter control. As species differences cannot be discussed in detail here, the reader is referred to recent reviews that discuss this and other species differences in detail (Andersson and Wein, 2004; Thor and de Groat, 2010; Andersson et al., 2011).

Despite the paucity of data on the development of urogenital innervation and mechanisms of its remodeling in adulthood, the importance of this area of the nervous system is illustrated by the many clinical problems that arise when the nervous system is perturbed. For example, various types of pelvic surgery performed for tumor removal unavoidably damage nearby nerves, resulting in incontinence and sexual dysfunction (Mauroy et al., 2003; Cornu et al., 2014; Wit and Horenblas, 2014; Laterza et al., 2015). Understanding factors that drive growth of these connections during development could identify strategies for their repair in adults. Conversely, chronic cystitis is accompanied by bladder hyperinnervation (Dickson et al., 2006; Boudes et al., 2013) that may contribute to hyperactivity and pain. In this case, identifying growth inhibitory or chemorepulsive factors involved in bladder nerve development may provide targets for reducing aberrant growth and dysfunction in adults.

The nerves of the bladder can be divided into two classes—sensory and autonomic—that differ in their anatomy, chemistry, and function (Jänig, 2008). There are multiple subtypes of sensory and autonomic nerves, as discussed below. As the bladder fills, the bladder wall relaxes to accommodate increasing volume and sympathetic nerves constrict the bladder base and urethra to promote continence. In cats, sympathetic innervation of the detrusor may contribute to continence (Vaughan and Satchell, 1992; Khadra et al., 1995) including by modulating transmission in parasym pathetic ganglion neurons.
Sensory Innervation of the Bladder

Sensory axons form a rich network in most bladder tissues (Gabella, 1995, 1999; Gabella and Davis, 1998; Forrest et al., 2014). They distribute uniformly in the smooth muscle (detrusor) of the bladder wall, but near the urothelium become heavily concentrated at the base and neck—where some axons also penetrate the deeper layers of urothelial cells (Gabella and Davis, 1998; Gillespie et al., 2006; Forrest et al., 2014). This suggests signaling from the urothelium to sensory nerves, which is important in both health and disease (Kanai, 2011; Birder and Andersson, 2013), is spatially restricted. Sensory axons are also found within many blood vessels in the bladder wall (Gabella and Davis, 1998; Forrest et al., 2014) where their role is unknown.

As in other internal organs, sensory axons in the bladder terminate in simple, free endings (Gabella, 1995, 1999; Gabella and Davis, 1998; Forrest et al., 2014). Although often regarded as structurally unspecialized, different branching patterns and terminal structures occur but these variations are relatively subtle in comparison with the large, complex structures at the terminals of specialized sensory nerves in skin and skeletal muscle (e.g., Pacinian and Ruffini corpuscles). These small structural variations have not yet been correlated with particular sensory modalities in the bladder.

Sensory axons in the bladder originate from distant neuronal cell bodies in the lumbar-sacral dorsal root ganglia (DRG) that lie close to each side of the spinal cord (Figure 1). After leaving the DRG, their axons pass through the pelvic autonomic ganglia en route to the bladder, then intermingle with the bladder-projecting motor axons until they reach the bladder wall. Sensory and motor axons cannot be distinguished at this point unless immunolabeled for specific neural markers. As most bladder sensory axons originate from the sacral DRG these neurons are better characterized and more commonly experimentally manipulated than those originating from upper lumbar DRG (Kanai, 2011; Gonzalez et al., 2014; de Groat and Yoshimura, 2015). Given only a minority of neurons in DRG of both spinal levels project to the bladder (Robinson and Gebhart, 2008; de Groat and Yoshimura, 2009), assessing their molecular changes during disease or injury states raises a technical challenge. Anatomical tracing strategies (e.g., retrograde tracer micro-injection into the bladder wall Yoshimura et al., 2003; Forrest et al., 2013; Gonzalez et al., 2014) have been critical for visualizing the bladder sensory neurons to define their properties.

Physiological studies recording electrical activity within single axons entering the bladder wall or at their terminals during different types of bladder manipulation (e.g., stretch) have subclassified them by transduction properties (Zagorodnyuk et al., 2007, 2010; Xu and Gebhart, 2008). The predominant class acts as low-threshold mechanosensors that respond to tension and contraction. However, other classes of low-threshold and high-threshold mecano- and chemo-sensitive nerves can be recruited, especially in response to physical or chemical signals of impending bladder damage (Kanai, 2011; Skryma et al., 2011). These latter signals may be interpreted as sensations of discomfort or pain, but normally the organism does not perceive activity in these neural pathways.

Bladder sensory nerves are either lightly myelinated (A-delta) fibers or unmyelinated (C-fibers) (Kanai, 2011; de Groat and Yoshimura, 2015). Most of the A-delta class comprises low-threshold mechanosensors whereas most C-type fibers are higher threshold sensors that respond to diverse types of stimuli (polymodal), including damage. Measurements of action potential conduction velocity distinguish these two classes, but molecular properties are also useful indicators. For example, the majority of C-fiber bladder sensory neurons express the nociceptive transducer ion channel, TRPV1 (transient receptor potential vanilloid receptor 1), as well as the neuropeptides, calcitonin gene-related peptide and substance P (Arms and Vizzard, 2011; Skryma et al., 2011; Forrest et al., 2013). Functional studies have also used capsaicin-sensitivity to identify activity attributed to bladder sensory neurons that express TRPV1 (de Groat and Yoshimura, 2009; Michel and Igawa, 2015). Conversely, the 200 kD neurofilament protein (NF200) distinguishes the cell bodies of sensory neurons with myelinated axons (Lawson et al., 1993), including the A-delta bladder afferents (Forrest et al., 2013). However, the latter has limited use in identifying their terminals where neurofilament levels are low (Forrest et al., 2014). Also, NF200 is not diagnostic of sensory axons in the bladder wherein many autonomic axons express this protein (Forrest et al., 2014).

Motor Innervation of the Bladder

The bladder can show myogenic activity that is independently driven by detrusor smooth muscle, but the effectors for the coordinated activity required by continence and micturition are parasympathetic and sympathetic autonomic neurons (Beckel and Holstege, 2011; de Groat and Yoshimura, 2015). Autonomic neurons also control vascular tone in the bladder (Michel and Igawa, 2015).

In the bladder, autonomic axons innervate the same tissues as the sensory axons, albeit in different density—in the detrusor and vasculature, autonomic axons are more prevalent than sensory axons, whereas the converse is true for the urothelium (Gabella and Davis, 1998; Gabella, 1999; Gillespie et al., 2006; Forrest et al., 2014). Although less prevalent than sensory axons, autonomic axons show a similar gradient of urothelial...
innervation toward the bladder base and neck (Dickson et al., 2006).

Parasympathetic axons release acetylcholine as a transmitter and in some tissues and species release a co-transmitter, ATP (Jänig, 2008). This is the dominant axon type in the detrusor, where they cause muscle contraction during micturition (de Groat and Yoshimura, 2015). Sympathetic axons release noradrenaline (norepinephrine) and potentially also ATP but are scarce in the detrusor, only being found in small numbers in the base and neck of the bladder where they have a role in continence (Kihara and de Groat, 1997; Gosling et al., 1999; Forrest et al., 2014; de Groat and Yoshimura, 2015). A small population of parasympathetic axons lies close to the urethra, where they are closely associated with sensory terminals (Dickson et al., 2006; Gillespie et al., 2006). The dominant population of sympathetic axons supplies the vasculature (Gabella, 1999; Forrest et al., 2014), where they likely cause constriction.

Most of the autonomic axons innervating the bladder originate from neuron cell bodies in the nearby pelvic ganglia (Figure 1) (Keast and de Groat, 1989). Sympathetic axons innervating the bladder vasculature most likely originate from neurons in the sympathetic chain (paravertebral) ganglia, as is the case for sympathetic vasoconstrictor axons in other organs (Jänig, 2008). The pelvic ganglia of rodents are functionally equivalent to the much more dispersed inferior hypogastric plexus in humans (Keast, 1999; Shoji et al., 2013). Rodent pelvic ganglia comprise paired clusters of thousands of neurons lying close to the uterine cervix and lateral lobes of the prostate, and contain many cell types. First, they contain neurons that each innervate a distinct region of the urogenital tract and lower bowel (Keast and de Groat, 1989; Kepper and Keast, 1995). Therefore, as discussed for identifying bladder sensory neurons in DRG, strategies such as retrograde tracing are valuable. Second, these target-defined neurons comprise both sympathetic and parasympathetic classes (Keast, 1995, 1999; Kanjhan et al., 2003; Jobling and Lim, 2008). Therefore, during development axons from two different regions of the spinal cord converge on the ganglia to synapse with the appropriate type of ganglion neuron. This mixture of sympathetic and parasympathetic neurons in one ganglion occurs nowhere else in the body. In addition to the pelvic ganglia, in rodents a few autonomic ganglion neurons are embedded in the bladder wall (Gillespie et al., 2006; Forrest et al., 2014), albeit are less common than intramural neurons in human bladder (Gilpin et al., 1983).

Development of Bladder Innervation

Sensory and autonomic axons innervating the bladder mostly originate from neuronal cell bodies that are some distance from the bladder tissues although some neuronal cell bodies appear transiently in the bladder wall during development and early postnatal life, with very few remaining by adulthood (Zvarova and Vizzard, 2005). Their properties more closely resemble autonomic than sensory neurons (Zvarova and Vizzard, 2005; Forrest et al., 2014) but their function is unknown. Therefore, to understand developmental mechanisms, it is necessary to identifying the relevant neuronal populations within DRG and pelvic ganglia. This includes defining the cues that promote growth and survival, determine guidance and appropriate synaptic targeting, and specify appropriate signaling properties (e.g., ion channel expression, transmitter synthesis). Progress in most of these areas remains limited. Bladder tissues express a typical array of neurotrophic factors (Vizzard, 2000; Vizzard et al., 2000; Gonzalez et al., 2014), and many neurotrophic factor receptors have been identified in neurons within the pelvic ganglia and bladder sensory neurons of the lumbosacral DRG (Murray et al., 2004; Hiltunen et al., 2005; Palma and Keast, 2006; Vizzard, 2006; Forrest et al., 2013; Gonzalez et al., 2014), but their roles in development of bladder innervation have not been defined.

Nerves grow into the bladder tissues well before birth. Axons enter the human fetal urinary bladder by 13 weeks post-conception and their density across each tissue type increases from that time (Kimmel and Mc, 1958; Klück, 1980; Gilpin et al., 1983; Dixon and Jen, 1995; Jen et al., 1995; Dixon et al., 1997, 1998, 1999). In these studies, immunohistochemistry for neuronal markers such as neuropeptides confirms that each of the major chemical classes of axons is likely to be present prior to birth. Moreover, neuronal cell bodies with transmitter chemistry similar to rodent pelvic ganglion neurons are embedded within the bladder wall and on its surface (Gilpin et al., 1983; Dixon et al., 1997); it is likely they are extensions of the inferior hypogastric plexus.

Our understanding of the timing and mechanisms of bladder innervation during embryonic development remains limited. In rodents, neural activation of the detrusor is evident just a few days after birth, but the properties of these contractions (including transmitter and receptor type) continue to change over the first couple of weeks (Levin et al., 1981; Maggi et al., 1984; Kruse and De Groat, 1990; Iuchi et al., 1994; Sann et al., 1997). Numerous images on the GUDMAP database (www.gudmap.org) (Harding et al., 2011) show that in embryonic mice, axons first reach the outer region of the detrusor by embryonic day (E) 14–15, and the urothelium by E18. At this time, axons can be distinguished by immunohistochemical markers that in adults would indicate parasympathetic, sympathetic and sensory axons. However, these may not be reliable markers in developing systems, where gene expression patterns may be quite dynamic. For example, tyrosine hydroxylase identifies adult noradrenergic sympathetic axons, but in development this enzyme is transiently expressed by a much broader neuron population (Rohrer, 2011). There is some early transient expression of cholinergic markers (Rohrer, 2011), and neuropeptide expression may be dynamic. Moreover, if expression of a particular neural marker is absent, it is tempting (but possibly inaccurate) to infer that the axon itself is not yet present.

Bladder-projecting sensory and autonomic neurons comprise only a minority of the neurons in pelvic ganglia and DRG, therefore studying their developmental mechanisms requires their identification (or separation) from surrounding neurons. Whereas, retrograde labeling can be conducted as simple recovery surgery in adult animals, comparable studies cannot be performed in the living embryo. Tracing nerve tracts in vitro by applying lipophilic dyes to fixed tissues (Ratchliffe et al., 2006) would be a valuable approach. This may also enable...
topographic maps to be constructed for developing bladder-projecting neurons, as performed previously for different types of neurons in pelvic ganglia of adult rats (Keast, 1999). In addition to correlating with expression patterns of transmitters, intriguingly expression patterns of transcription factors have been reported in developing mouse pelvic ganglia (Wiese et al., 2012), some of which may correlate with bladder-specific pathways. In parallel with the order of organ maturation, is also possible that bladder-projecting pelvic ganglion neurons differentiate and mature much earlier than those innervating reproductive organs. Therefore, to determine the mechanism by which the bladder becomes innervated, a range of ganglion systems investigated and the neurons within those ganglia that project to bladder identified. Immunohistochemical tools enable distinction of some elements within this system, but without a specific molecular phenotype yet identified for bladder-projecting neurons, progress in defining the route and source of connections will be constrained. Understanding development of mechanisms by which nerves communicate with non-neuronal cells within the bladder, such as glial cells and interstitial cells (Gabella, 1995, 1999; McCloskey, 2011) is also important.

**Genetic Models of Bladder Dysfunction**

Animal models provide valuable insights into the factors contributing to particular bladder phenotypes and the mechanisms driving development of normal bladder innervation. Here we highlight some examples of mouse models with dysfunction due to enlarged bladders. We will discuss these in the context of possible neuronal impairment derived from loss of sensory transduction (to detect distension) or motor control (to enable contraction and emptying).

Deletion of the nicotinic acetylcholine receptor Chrna3, or the combined deletion of Chrnb2 and Chrna4, result in a bladder phenotype known as megacystis (Xu et al., 1999a,b). This is characterized by extreme bladder enlargement, overflow incontinence and bladder infection with urinary stones. These features are likely driven by neuronal dysfunction, as nicotinic receptors are expressed by autonomic ganglion neurons and required for transmitting the excitatory message from spinal neurons to ganglion cells, and hence to the organs. Specifically, Chrna3 and Chrnb4 are highly expressed in pelvic ganglia (Park et al., 2006; Girard et al., 2013) and are upregulated in parasympathetic pelvic ganglion neurons in a surgical obstruction model of the urethra (Chung et al., 2015). While not excluding other mechanisms, these reports are consistent with the bladder dysfunction being driven by an inability to void, resulting in distension and hypertrophy of the bladder. This model could provide additional insights into the impact of autonomic inactivity (rather than neuronal loss) on upstream components of the reflex circuitry. The effects of prolonged distension on sensory neurons could reveal mechanisms relevant to a number of clinical obstructive conditions.

A more bladder-specific disruption has been seen with “megabladder” mice (Singh et al., 2007; McHugh, 2014), a phenotype caused by randomly inserting a transgene into chromosome 16 that subsequently, along with a portion of chromosome 16, translocated to chromosome 11 downstream of myocardin. Profound changes, including severe distension and thinning of bladder wall were reported in utero, with the bladder almost completely lacking detrusor muscle; less dramatic changes occur in lamina propria and urothelium. This severe problem was restricted to lower urinary tract; muscle development was normal in the gastrointestinal and respiratory tracts, and vascular system. The innervation of these bladder tissues has not yet been examined but could reveal if and how a normal detrusor determines normal bladder innervation patterns. Furthermore, the phenotype was more severe in male, providing a platform to study sex differences in neuronal development.

A different outcome occurred after deleting FGFR2 (fibroblast growth factor receptor 2) from the bladder mesenchyme, leading to thinner detrusor muscle but in this case a thickened, collagen-enriched lamina propria (Walker et al., 2015). This impacted on muscle contractility in response to cholinoreceptor and purinoceptor agonists and compliance of the bladder wall. Innervation density was not examined, but their decreased intervoiding interval in cystometry studies provides further encouragement to examine sensory nerve density and threshold for activation in these animals.

**Conclusions**

This review highlights the primary features of bladder innervation in the adult in order to demonstrate the gaps to be filled in understanding the development of this anatomically and functionally complex nerve supply. Defining the developmental mechanisms in this system may also reveal strategies to drive regeneration, targeting and functional recovery of bladder nerves in the adult.

**Acknowledgments**

This work was supported by the National Institutes of Health (S01 DK094479 to JK) and the National Health and Medical Research Council of Australia (1003512 to PO and JK, 1022941 to JK).

**References**

Andersson, K. E., Soler, R., and Füllhase, C. (2011). Rodent models for urodynamic investigation. Neurourol. Urodyn. 30, 636–646. doi: 10.1002/nau.21108

Andersson, K. E., and Wein, A. J. (2004). Pharmacology of the lower urinary tract: basis for current and future treatments of urinary incontinence. Pharmacol. Rev. 56, 581–631. doi: 10.1124/pr.56.4.4

Arms, L., and Vizzard, M. A. (2011). Neuropeptides in lower urinary tract function. *Handb. Exp. Pharmacol. 202*, 395–423. doi: 10.1007/978-3-642-16499-6_19
Beckel, J. M., and Holstege, G. (2011). Neurophysiology of the lower urinary tract. *Handb. Exp. Pharmacol.*, 202, 149–169. doi: 10.1007/978-3-642-16499-6_8

Bider, L., and Anderson, K. E. (2013). Urothelial signaling. *Physiol. Rev.*, 93, 653–680. doi: 10.1152/physrev.00350.2012

Boudes, M., Uvin, P., Pinto, S., Freichel, M., Bunzhauser, L., Voets, T., et al. (2013). Crucial role of TRPC1 and TRPC4 in cysitides-induced neuronal sprouting and bladder overactivity. *PLoS ONE* 8, e69550. doi: 10.1371/journal.pone.0069550

Chung, H. C., Lee, C. K., Park, K. H., and Jeong, S. W. (2015). Bladder outlet obstruction causes up-regulation of nicotinic acetylcholine receptors in bladder-projecting pelvic ganglion neurons. *Brain Res.* 1602, 111–118. doi: 10.1016/j.brainres.2015.01.026

Cornu, J. N., Melot, C., and Haab, F. (2014). A pragmatic approach to the characterization and effective treatment of male patients with postprostatectomy incontinence. *Curr. Opin. Urol.*, 24, 566–570. doi: 10.1097/MOU.0000000000000112

De Groat, W. C., and Saum, W. R. (1972). Sympathetic inhibition of the urinary bladder. *J. Physiol.* 230, 615–637. doi: 10.1113/jphysiol.1972.sp005955

De Groat, W. C., and Yoshimura, N. (2009). Afferent nerve regulation of bladder function in health and disease. *Handb. Exp. Pharmacol.*, 194, 91–138. doi: 10.1007/978-3-540-79909-7_4

Dixon, J. S., Jen, P. Y., and Gosling, J. A. (1999). The distribution of noradrenergic neurones in the human lower urinary tract. A review. *Eur. Urol.* 36(Suppl. 1), 23–30. doi: 10.1109/000052314

Gonzalez, E. J., Merrill, L., and Vizzard, M. A. (2014). Bladder sensory physiology: neuroactive compounds and receptors, sensory transducers, and target-derived growth factors as targets to improve function. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 306, R869–R878. doi: 10.1152/ajpregu.00030.2014

Gosling, J. A., Dixon, J. S., and Jen, P. Y. (1999). The distribution of noradrenergic neurones in the human lower urinary tract. *Am. J. Physiol.* 277(6), R1504–R1512. doi: 10.1152/ajpregu.00343.2013

Gonzalez, E. J., Merrill, L., and Vizzard, M. A. (2014). Bladder sensory physiology: neuroactive compounds and receptors, sensory transducers, and target-derived growth factors as targets to improve function. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 306, R869–R878. doi: 10.1152/ajpregu.00030.2014

Keast, J. R. (1995). Visualization and immunohistochemical characterization of sympathetic and parasympathetic neurones in the male rat pelvic ganglion. *Neuroscience* 66, 653–662. doi: 10.1016/0306-4522(94)00595-V

Keast, J. R. (1999). Unusual autonomic ganglia: connections, chemistry, and plasticity of pelvic ganglia. *Int. Rev. Cytol.* 193, 1–69. doi: 10.1007/978-7-6796-0816778-7

Klück, P. (1980). The autonomic innervation of the human urinary bladder, its function in health and disease. *Anat. Embryol. (Berl.)* 161, 157–160. doi: 10.1007/BF00417871

Klück, P. (1980). The autonomic innervation of the human urinary bladder, its function in health and disease. *Anat. Embryol. (Berl.)* 161, 157–160. doi: 10.1007/BF00417871

Kruse, M. N., and De Groat, W. C. (1990). Micturition reflexes in de cerebrate and spinal cats. *J. Neurophysiol.* 63, 149–169. doi: 10.1152/jn.00479.2002

Kucharczyk, C., and Kruse, M. N. (2013). Sympathetic control of pelvic ganglion neurons in female mice. *Auton. Neurosci.* 140, 30–39. doi: 10.1016/j.autneu.2008.03.001

Kucuk, A. I. (2011). Afferent mechanism in the urinary tract. *Handb. Exp. Pharmacol.* 202, 171–205. doi: 10.1007/978-3-642-16499-6_9

Kübler, P., and Keast, J. R. (2004). Postnatal maturational changes in rat pelvic autonomic ganglion cells: a mixture of steroid-dependent and -independent effects. *J. Neurophysiol.* 89, 315–323. doi: 10.1152/jn.00747.2002

Keast, J. R. (1995). Visualization and immunohistochemical characterization of sympathetic and parasympathetic neurones in the male rat pelvic ganglion. *Neuroscience* 66, 653–662. doi: 10.1016/0306-4522(94)00595-V

Keast, J. R. (1999). Unusual autonomic ganglia: connections, chemistry, and plasticity of pelvic ganglia. *Int. Rev. Cytol.* 193, 1–69. doi: 10.1007/978-7-6796-0816778-7

Keast, J. R., and De Groat, W. C. (1989). Immunohistochemical characterization of pelvic neurones which project to the bladder, colon, or penis in rats. *J. Comp. Neurol.* 288, 387–400. doi: 10.1002/cne.920880300

Kil сделать, M. N., and Keast, J. R. (2005). Immunohistochemical properties and spinal connections of pelvic autonomic neurones that innervate the rat prostate gland. *Cell Tissue Res.* 281, 533–542. doi: 10.1007/BF00417781

Kruse, M. N., and De Groat, W. C. (2000). Micturition reflexes in de cerebrate and spinalized neonatal rats. *Am. J. Physiol.* 285, R1508–R1511

Laterza, R. M., Sievert, K. D., de Ridder, D., Vierhout, M. E., Haab, F., Cardozo, L., et al. (2015). Bladder function after radical hysterectomy for cervical cancer. *Neurourol. Urodyn.* 34, 309–315. doi: 10.1002/nau.22570

Lawson, S. N., Perry, M. J., Prabhakar, E., and McCarthy, P. W. (1993). Primary sensory neurones in the context of myelination, receptors for pain modulators, neuroactive compounds and receptors, sensory transducers, and target-derived growth factors as targets to improve function. *Am. J. Physiol.* 270, 2845–2853. doi: 10.1152/dev.063594

Laterza, R. M., Sievert, K. D., de Ridder, D., Vierhout, M. E., Haab, F., Cardozo, L., et al. (2015). Bladder function after radical hysterectomy for cervical cancer. *Neurourol. Urodyn.* 34, 309–315. doi: 10.1002/nau.22570

Levin, R. M., Malkowicz, S. B., Jacobowitz, D., and Wein, A. J. (1981). The ontogeny of the autonomic innervation and contractile response of the rabbit urinary bladder. *J. Pharmacol. Exp. Ther.* 219, 250–257.

Maggi, C. A., Santi, D., and Melli, A. (1984). Postnatal development of myogenic contractile activity and excitatory innervation of rat urinary bladder. *Am. J. Physiol.* 247, R972–R978.
Mauroy, B., Demondion, X., Drizenko, A., Gouillet, E., Bonnal, J.-L., Bieserte, J., et al. (2003). The inferior hypogastric plexus (pelvic plexus): its importance in neural preservation techniques. Surg. Radiol. Anat. 25, 6–15. doi: 10.1007/s00276-002-0083-3

McCloskey, K. D. (2011). Interstitial cells and bladder pathophysiology—passive bystanders or active participants? J. Urol. 185, 1562–1563. doi: 10.1016/j.juro.2011.02.2587

McHugh, K. M. (2014). Megabladder mouse model of congenital obstructive nephropathy: genetic etiology and renal adaptation. Pediatr. Nephrol. 29, 645–650. doi: 10.1007/s00467-013-2658-6

Michel, M. C., and Iagawa, Y. (2015). Therapeutic targets for overactive bladder other than smooth muscle. Expert Opin. Ther. Targets 19, 687–705. doi: 10.1517/14728222.2015.1009447

Murray, E., Malley, S. E., Qiao, L. Y., Hu, V. Y., and Vizzard, M. A. (2004). Cyclophosphamide induced cystitis alters neurotrophin and receptor tyrosine kinase expression in pelvic ganglia and bladder. J. Urol. 172, 2434–2439. doi: 10.1097/01.ju.0000143549.29867.4e

Palma, C. A., and Keast, J. R. (2006). Structural effects and potential changes in growth factor signalling in pennis-projecting autonomic neurons after axotomy. BMC Neurosci. 7:41. doi: 10.1186/1471-2202-7-41

Park, K. S., Cha, S. K., Kim, M. J., Kim, D. R., Jeong, S. W., Lee, J. W., et al. (2006). An alpha3beta4 subunit combination acts as a major functional nicotinic acetylcholine receptor in rat pelvic ganglion neurons. Pflugers Arch. 452, 773–783. doi: 10.1007/s00424-006-0086-1

Ratcliffe, E. M., Setru, S. U., Chen, J. J., Li, Z. S., D’Autreux, F., and Gershon, M. D. (2006). Netrin/DEC-mediated attraction of vagal sensory axons to the fetal mouse gut. J. Comp. Neurol. 498, 567–580. doi: 10.1002/cne.21027

Robinson, D. R., and Gebhart, G. F. (2008). Inside information: the unique features of visceral sensation. Mol. Interv. 8, 242–253. doi: 10.1124/mi.8.5.9

Rohrer, H. (2011). Transcriptional control of differentiation and neurogenesis in autonomic ganglia. Eur. J. Neurosci. 34, 1563–1573. doi: 10.1111/j.1460-9568.2011.07860.x

Sann, H., Walb, G., and Pierau, F. K. (1997). Postnatal development of the autonomic and sensory innervation of the musculature in the rat urinary bladder. Neurosci. Lett. 236, 29–32. doi: 10.1016/S0304-3990(97)00752-0

Shojai, M. M., Sharma, A., Mirzayan, N., Groat, C., Watanabe, K., Loukas, M., et al. (2013). Neuroanatomy of the female abdominopelvic region: a review with application to pelvic pain syndromes. Clin. Anat. 26, 66–76. doi: 10.1002/ca.22200

Singh, S., Robinson, M., Nahi, F., Coley, B., Robinson, M. L., Bates, C. M., et al. (2007). Identification of a unique transgenic mouse line that develops megabladder, obstructive uropathy, and renal dysfunction. J. Am. Soc. Nephrol. 18, 481–491. doi: 10.1681/ASN.2006040405

Skryma, R., Prevarskaya, N., Gkika, D., and Shuba, Y. (2011). From urgency to frequency: facts and controversies of TRPs in the lower urinary tract. Nat. Rev. Urol. 8, 617–630. doi: 10.1038/nruro.2011.142

Thor, K. B., and de Groat, W. C. (2010). Neural control of the female urethral and anal rhabdosphincters and pelvic floor muscles. Am. J. Physiol. Regul. Integr. Comp. Physiol. 299, R416–R438. doi: 10.1152/japplregu.00111.2010

Vaughan, C. W., and Satchell, P. M. (1992). Role of sympathetic innervation in the feline continence process under natural filling conditions. J. Neurophysiol. 68, 1842–1849.

Vizzard, M. A. (2000). Changes in urinary bladder neurotrophic factor mRNA and NGF protein following urinary bladder dysfunction. Exp. Neurol. 161, 273–284. doi: 10.1006/exnr.1999.7254

Vizzard, M. A. (2006). Neurochemical plasticity and the role of neurotrophic factors in bladder reflex pathways after spinal cord injury. Prog. Brain Res. 152, 97–115. doi: 10.1016/S0079-6123(05)52007-7

Vizzard, M. A., Wu, K. H., and Jewett, I. T. (2000). Developmental expression of urinary bladder neurotrophic factor mRNA and protein in the neonatal rat. Brain Res. Dev. Brain Res. 119, 217–224. doi: 10.1016/S0165-3806(99)00174-1

Walker, K. A., Ikeda, Y., Zabbarova, I., Schaefer, C. M., Bushnell, D., De Groat, W. C., et al. (2015). Fgf2 is integral for bladder mesenchyme patterning and function. Am. J. Physiol. Renal. Physiol. 308, F888–F898. doi: 10.1152/ajprenal.00624.2014

Wiese, C. B., Ireland, S., Fleming, N. L., Yu, J., Valerius, M. T., Georgas, K., et al. (2012). A genome-wide screen to identify transcription factors expressed in pelvic Ganglia of the lower urinary tract. Front. Neurosci. 6:130. doi: 10.3389/fnins.2012.00130

Wit, E. M., and Horenblad, S. (2014). Urological complications after treatment of cervical cancer. Nat. Rev. Urol. 11, 110–117. doi: 10.1038/nrurol.2013.323

Xu, L., and Gebhart, G. F. (2008). Characterization of mouse lumbar splanchic and pelvic nerve urinary bladder mechano sensory afferents. J. Neurophysiol. 99, 244–253. doi: 10.1152/jn.0049.2007

Xu, W., Gelber, S., Orr-Urtreger, A., Armstrong, D., Lewis, R. A., Ou, C. N., et al. (1999a). Megacystis, mydriasis, and ion channel defect in mice lacking the alpha3 neuronal nicotinic acetylcholine receptor. Proc. Natl. Acad. Sci. U.S.A. 96, 5746–5751. doi: 10.1073/pnas.96.10.5746

Xu, W., Orr-Urtreger, A., Nigro, F., Gelber, S., Sutcliffe, C. B., Armstrong, D., et al. (1999b). Multigang autonomic dysfunction in mice lacking the beta2 and the beta3 subunits of neuronal nicotinic acetylcholine receptors. J. Neurosci. 19, 9298–9305.

Yoshimura, N., Seki, S., Erickson, K. A., Erickson, V. L., Hancoll, M. B., and de Groat, W. C. (2003). Histological and electrical properties of rat dorsal root ganglion neurons innervating the lower urinary tract. J. Neurosci. 23, 4355–4361.

Zagorodnyuk, V. P., Brookes, S. J., and Spencer, N. J. (2010). Structure-function relationship of sensory endings in the gut and bladder. Auton. Neurosci. 153, 3–11. doi: 10.1016/j.autneu.2009.07.018

Zagorodnyuk, V. P., Gibbins, I. L., Costa, M., Brookes, S. J., and Gregory, S. J. (2007). Properties of the major classes of mechanoreceptors in the guinea pig bladder. J. Physiol. 585, 147–163. doi: 10.1113/jphysiol.2007.140244

Zvarova, K., and Vizzard, M. A. (2005). Distribution and fate of cocaine- and amphetamine-regulated transcript peptide (CARTp)-expressing cells in rat urinary bladder: a developmental study. J. Comp. Neurol. 489, 501–517. doi: 10.1002/cne.20657

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2015 Keast, Smith-Anttila and Osborne. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.
