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Editor's Summary

Getting to the Root of Bladder Control

Injury to the spinal cord typically results in loss of conscious bladder emptying and the sensation of fullness. Currently, only limited treatment options are available, with most of the patients receiving catheterization. However, this is cumbersome and leads to urological complications including unsolicited episodes of bladder contraction, leading to inappropriate emptying. In a new study, Chew *et al.* design a ''closed-loop'' electronic device that can accurately record bladder filling from sensory nerves after spinal cord injury in rat. Using this information, bladder emptying can be artificially stimulated on demand by electrically modulating nerve firing. It is traditionally difficult to record robust neuronal activity from peripheral nerves in vivo. Typically, cuff electrodes are used to record from whole nerves, but produce poor signal quality and provide little indication of bladder filling. Through nerve microdissection, Chew *et al.* implanted fine-diameter nerves (''rootlets'') into insulated microchannels, recording action potential firing that accurately encoded bladder filling. The device had multiple microchannels for concurrent recording, greatly improving the resolution. Using this sensory information and by manipulating stimulation characteristics, the authors prevented the rat bladder from emptying inappropriately, and bladder contraction was initiated when desired. This work opens a new avenue for the design of a neuroprosthesis for bladder control after spinal cord injury.

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NEUROPROSTHETICS

A Microchannel Neuroprosthesis for Bladder Control After Spinal Cord Injury in Rat

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A severe complication of spinal cord injury is loss of bladder function (neurogenic bladder), which is characterized by loss of bladder sensation and voluntary control of micturition (urination), and spontaneous hyperreflexive voiding against a closed sphincter (detrusor-sphincter dyssynergia). A sacral anterior root stimulator at low frequency can drive volitional bladder voiding, but surgical rhizotomy of the lumbosacral dorsal roots is needed to prevent spontaneous voiding and dyssynergia. However, rhizotomy is irreversible and eliminates sexual function, and the stimulator gives no information on bladder fullness. We designed a closed-loop neuroprosthetic interface that measures bladder fullness and prevents spontaneous voiding episodes without the need for dorsal rhizotomy in a rat model. To obtain bladder sensory information, we implanted teased dorsal roots (rootlets) within the rat vertebral column into microchannel electrodes, which provided signal amplification and noise suppression. As long as they were attached to the spinal cord, these rootlets survived for up to 3 months and contained axons and blood vessels. Electrophysiological recordings showed that half of the rootlets propagated action potentials, with firing frequency correlated to bladder fullness. When the bladder became full enough to initiate spontaneous voiding, high-frequency/amplitude sensory activity was detected. Voiding was abolished using a high-frequency depolarizing block to the ventral roots. A ventral root stimulator initiated bladder emptying at low frequency and prevented unwanted contraction at high frequency. These data suggest that sensory information from the dorsal root together with a ventral root stimulator could form the basis for a closed-loop bladder neuroprosthetic.

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INTRODUCTION

After spinal cord injury, many patients develop a neurogenic bladder with loss of conscious control of micturition (urination). Normal micturition depends on coordinated bladder contraction and sphincter relaxation controlled by the lumbosacral neuronal circuitry, and is initiated and inhibited by descending control from the brainstem (*1*, *2*). Spinal cord injury rostral to the lumbosacral lower motor neuron pool eliminates voluntary control of voiding. Acutely, this results in an areflexic bladder and urinary retention, rapidly leading to life-threatening infection if not treated. Slowly, an automatic micturition pattern can emerge from plasticity within the lumbosacral neuronal circuitry (*3*). However, this is not under conscious sensation or control, and patients experience short intermittent reflexive voiding, leading to frequent episodes of incontinent dribbling, and detrusor-sphincter dyssynergia (DSD) as the bladder contracts against a closed sphincter. Regaining bladder control is among the principal desires of patients presenting with spinal cord injury (*4*). There are currently two options for bladder control. The simplest is paralysis of the bladder with botulinum toxin accompanied by regular catheterization. Alternatively, many patients worldwide have been equipped with the Finetech-Brindley sacral anterior root stimulator (SARS), which enables elective emptying of the bladder through stimulation of the sacral anterior/ventral roots that innervate the detrusor muscle (*5*–*10*). Recently, we have implanted several newly designed SARS devices into dogs, providing a good clinical solution for their incontinence (*11*). Although bladder emptying is reliable, the limitation of the SARS device for human patients is the requirement for multiple rhizotomies (transections) of the posterior/dorsal roots to prevent hyperreflexive bladder emptying, and to relax the external urethral sphincter to prevent DSD. However, this entails loss of reflexive erectile function, loss of vaginal lubrication, and weakening of the pelvic floor muscles (*8*, *12*). This has persuaded many patients and surgeons to choose catheterization coupled with bladder paralysis using botulinum toxin. However, most of the patients with spinal cord injury would still prefer improved neural prostheses (*13*). We have therefore sought to develop a solution that will enable the use of a SARS for bladder emptying while preserving the function of the sensory roots (Fig. 1). The main requirement is to abort episodes of spontaneous bladder emptying so that elective emptying can then be achieved using a SARS. To make this possible, recording of sensory information is necessary to detect bladder fullness and episodes of bladder contraction. This must be coupled with a method to block unwanted contractions.

Recording action potentials from axons is difficult because small currents that pass across the axon membrane exit into a very low resistance extracellular space, leading to low-voltage potential ($V = IR$, Ohm's law) and poor signal-to-noise ratio (SNR). In acute recording, the problem is solved by surrounding nerves with high electrically resistant oil, but this is not practical for clinical implant application. Current designs for peripheral nerve recording interfaces have various issues that

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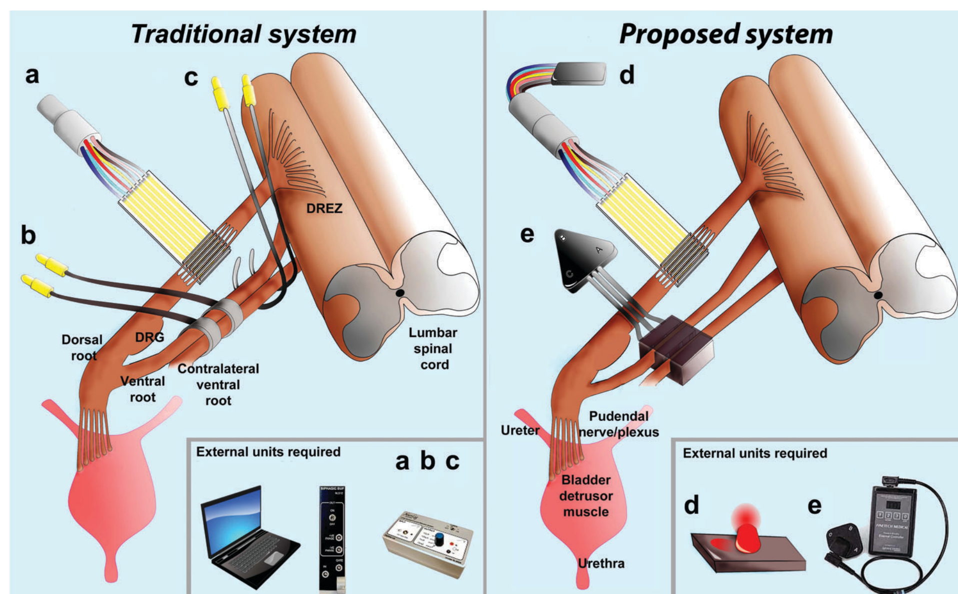

Fig. 1. Design of a closed-loop neuroprosthesis for bladder control. A requirement for adequate closed-loop control of micturition is the detection of bladder fullness and spontaneous emptying. Bladder fullness and emptying can be recorded from teased spinal cord dorsal roots placed within a microchannel-electrode interface (a). Low-frequency stimulation of the ventral roots causes bladder emptying (b). High-frequency depolarizing block prevents unsolicited bladder hyperreflexive emptying (c). The current recording systems required to perform this recording and stimulation preparation are shown in the left panel and are not suitable for human application. A potential modification for clinical use is shown in the right panel. We envisage that subcutaneously implanted amplifying and filtering equipment (telemeter) would monitor the bladder afferent firing rate to determine bladder fullness and detect spontaneous emptying. This telemeter would then be probed externally by a wireless receiver (d). Once visual or auditory indication has been given that the bladder is filling (d), and using an adapted stimulator unit (e), the user could initiate bladder emptying through low-frequency stimulation. If the device detected spontaneous emptying, a high-frequency conduction block would be applied until a suitable time and location could be found for emptying the bladder through traditional low-frequency stimulation. DRG, dorsal root ganglion.

have prevented routine clinical use. These include regenerative sieve electrodes (*14*–*16*) that require transection of a peripheral nerve for implantation, invasive penetrative arrays for intracellular recording from dorsal root ganglia (*17*–*19*), and cuff electrodes that wrap around intact sensory nerves and roots (*20*–*23*) but generally produce a poor SNR even with optimal filtering (*24*). For example, recording of bladder sensory information has been attempted using cuffs in anesthetized humans (*21*), but the signal size (5 μV, SNR = 0.2) is insufficient for use while awake and moving. Techniques under development include velocity-selective recordings from multielectrode cuffs to generate a more detailed action potential profile (*25*, *26*), but these currently require a very high sampling rate and detailed offline analysis (*27*).

Here, we have produced a new design for a nerve-electrode interface that combines features of both sieve and cuff designs by confining axons in 100-μm-diameter microchannels. The microchannels greatly increase extracellular resistance, thus amplifying recordable voltage potential. Inclusion of guard/reference electrodes at the ends of the microchannels suppresses noise interference (*28*, *29*). We have further developed this to produce implantable neuroprosthetic devices with encapsulated electrodes to record cutaneous (*30*) and bladder activity in acutely teased dorsal roots of rats (*31*). Dorsal root anatomy is fascicular, enabling teasing into multiple 100-μm fascicles/rootlets for implantation into microchannels. To preserve sensory function for the patient (*32*), this implantation can be accomplished without cutting the root from the cord (rhizotomy). We implanted microchannel electrodes on teased L6 dorsal rootlets of rats, selected because they contain 84% of total bladder afferents (*33*). These rootlets survived and were functional for at least 3 months after implantation. About half of the rootlets carried spike waveforms that correlated with bladder filling and emptying. To prevent hyperreflexive micturition, we successfully induced high-frequency depolarizing conduction blockade in the motor nerves innervating the bladder detrusor muscle. We suggest that it might now be possible to design a closed-loop neuroprosthesis for bladder control that does not involve dorsal rhizotomy.

RESULTS

Our goal was to demonstrate the techniques necessary for closed-loop bladder control for spinal cord injury while preserving the dorsal root connection to the spinal cord. To prevent unsolicited spontaneous emptying, one must extract sensory signals that indicate bladder contractions and, ideally, also measure bladder filling. A method is then needed to prevent spontaneous bladder emptying. We addressed these requirements by microchannel sensory recording from dorsal roots and conduction block through high-frequency stimulation to the ventral roots.

Dorsal root dissection and insertion into microchannels

Polydimethylsiloxane (PDMS) microchannel devices of 100 μm × 100 μm × 3 mm (Fig. 2, A and B) were implanted into the rat intradural space. Dorsal roots could readily be teased into 5 to 10 fine rootlets of about 100-μm diameter using glass dissecting probes. This was aided by arrangement of the roots into parallel fascicles and minimal presence of the thick outer epineurial sheath found typically surrounding peripheral nerves (Fig. 2C). In most of our experiments, the rootlets remained attached to the spinal cord (non-rhizotomized); in some, the root was cut at the entry zone (rhizotomized). A potential concern was that blood supply might be interrupted by dissection of the rootlets. However, small intact blood vessels (identified by red blood cells) could be observed in most of the rootlets after teasing. The teased rootlets were placed in microchannels, which were then closed by a PDMS lid (Fig. 2D). The L6 dorsal root was reliably identified by the L1 vertebral level and dorsal root entry zone (DREZ) (Fig. 2E). Implants integrated well on top of the spinal cord because they were only 300 μm in total depth (Fig. 2F). Subsequent reexposure at later time points was straightforward, and rootlets were functionally assessed using hook electrodes and electrophysiology equipment (Fig. 2G).

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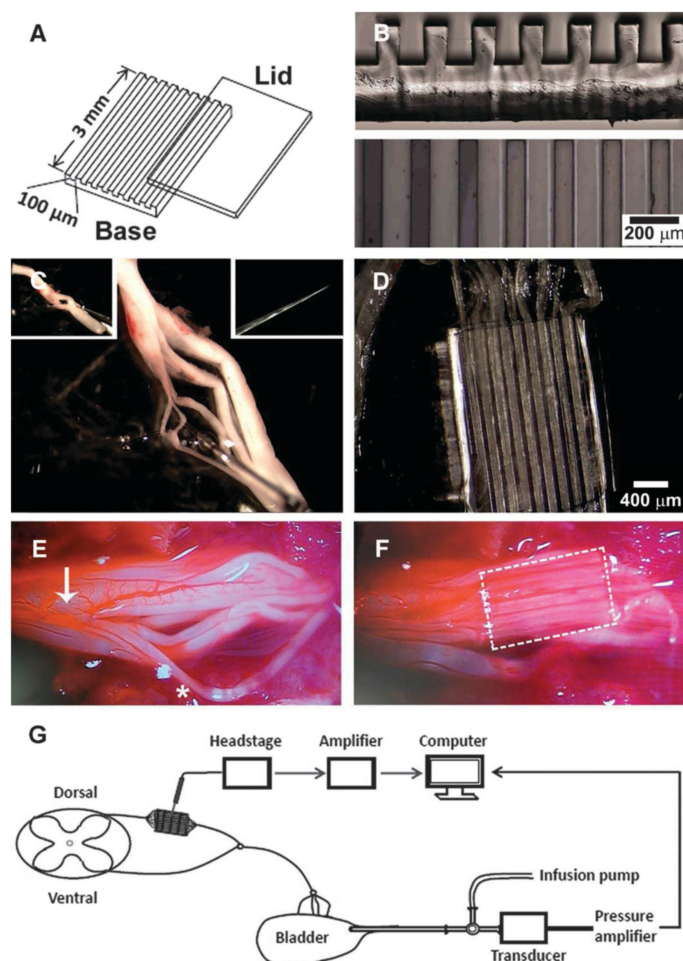

Fig. 2. Surgical implantation of teased rat dorsal roots into PDMS microchannels. (**A** and **B**) A PDMS base (200-μm thick) containing up to ten 100-μm^2 microchannels, covered by a PDMS lid, constitutes the device. (**C**) Surgical manipulation of the dorsal root involves teasing the whole root (inset) with glass pulled probes (inset) into fascicles or "rootlets" of ~100 μm in diameter. (**D**) These are then laid into the microchannels preceding lid placement. (**E**) The L6 dorsal root (asterisk) can be identified from the vertebral level (L1-L2) and from the DREZ (white arrow). (**F**) The microchannel implant is laid on the dorsal surface of the S1-S2 spinal cord (dashed box). (**G**) During a recording, the bladder is catheterized and connected to an infusion pump and a pressure transducer, and the implant is reexposed. Rootlets are probed using surface contact silver hook electrodes under oil.

Survival of dorsal root axons in microchannels

Anatomical survival of rootlets in microchannels was assessed. Tissue remained visible in the microchannels at 3 months, even in rhizotomized animals (Fig. 3A). Rootlets removed from microchannels were sectioned at 1 week, 1 month, and 3 months after implantation (Fig. 3B). By 1 week, most of the non-rhizotomized rootlets contained at least one blood vessel positive for the expression of rat endothelial cell antigen–1 (RECA-1); this was maintained at 3 months. Conversely, no blood vessels were found at 3 months in rhizotomized rootlets, although some had hollow areas in the rootlet reminiscent of necrotic vessels. In non-rhizotomized rootlets, myelination [indicated by the expression of myelin basic protein (MBP)] was significantly diminished at 1 week (9.79 ± 0.50%) after dissection compared to rootlets assessed immediately after teasing (35.5 ± 1.58%) ($F_{4,20}$ = 98.1, P < 0.001), but there was no further decrease over 3 months (10.6 ± 1.59%) (Fig. 3C). Rhizotomized rootlets, however, were almost devoid of myelin by 3 months (0.18 ± 0.16%), significantly less than nonrhizotomized counterparts (P < 0.001). Non-rhizotomized rootlets demonstrated good axon survival (as indicated by β_3-tubulin expression), with no significant difference between immediate teasing and 1 week of implantation (12.4 ± 2.11); the axon number did not significantly decrease at 3 months (9.6 ± 1.47). However, rhizotomized rootlets showed almost complete axonal loss at 3 months (1.20 ± 0.58 axons per section) when compared to immediately after teasing (16.20 ± 2.50) ($F_{4,20}$ = 8.936, P < 0.001) and to nonrhizotomized rootlets (P < 0.05) (Fig. 3D). These results show that the trauma of surgery leads to initial loss of myelin, but not axons. Microchannel encapsulation over 3 months does not lead to further degeneration of axons or myelin. However, if the rootlets are rhizotomized, severe demyelination and axonal loss are observed.

Effects of dorsal root surgery on the bladder

To ascertain whether unilateral rhizotomy of L6 bladder afferents or insertion of a microchannel interface might affect the ability of the bladder to empty normally, we measured bladder volume in vivo from width, length, and depth measurements using ultrasound (fig. S2A). There was no evidence of bladder distension, indicating urine retention, up to 3 months after surgery (63 ± 18 mm^3) compared to controls (54 ± 9 mm^3) [two-tailed $t(6)$ = 0.43, P = 0.68] (fig. S2B). Bladder diameter was also measured postmortem (fig. S2C), and again, no significant increase in bladder size was shown up to 3 months (5.2 ± 0.03 mm) (fig. S2D) compared to control (4.2 ± 0.29 mm) [two-tailed $t(6)$ = 2.41, P > 0.05].

Acute recordings from teased dorsal roots in microchannels

Having shown that implanted rootlets can survive within the microchannels, we assessed their ability to conduct action potentials that provide reliable information on bladder fullness and emptying. First, rootlets placed in "passive" (without inbuilt electrodes) microchannels were probed using silver hook electrodes immersed in oil. This is the optimum method for extracellular axon recording, with very high extracellular resistance and therefore large action potentials, but is not practical for chronic recording. These results were then compared with recordings taken from "active" microchannels with inbuilt electrodes.

Hook and cuff electrodes. During bladder infusion, evoked action potential recordings were first taken from the whole root using cuff and hook electrodes. After this, recordings were taken from sequentially teased rootlets with gradually decreasing diameter to demonstrate the baseline electrophysiology of rootlets immediately after dissection and the rationale for the teasing method (Fig. 4). Recording from the whole unteased L6 dorsal root produced very poor SNR during bladder filling using either cuff electrodes in saline or hook electrodes in oil, and only cutaneous afferent activity could be detected. Much clearer recordings with lower background noise could be obtained from microdissected rootlets. Sequential dissections (half, quarter, eighth) produced corresponding stepwise improvements in SNR (Fig. 4A). Some recorded waveforms showed an increase in spike frequency with increasing infusion volume. These were around 100 μV in amplitude as the bladder filled up to around 0.4 ml, at a pressure of 5 to 10 cm of water (cm H_2O) (Fig. 4B). These volumes and pressures

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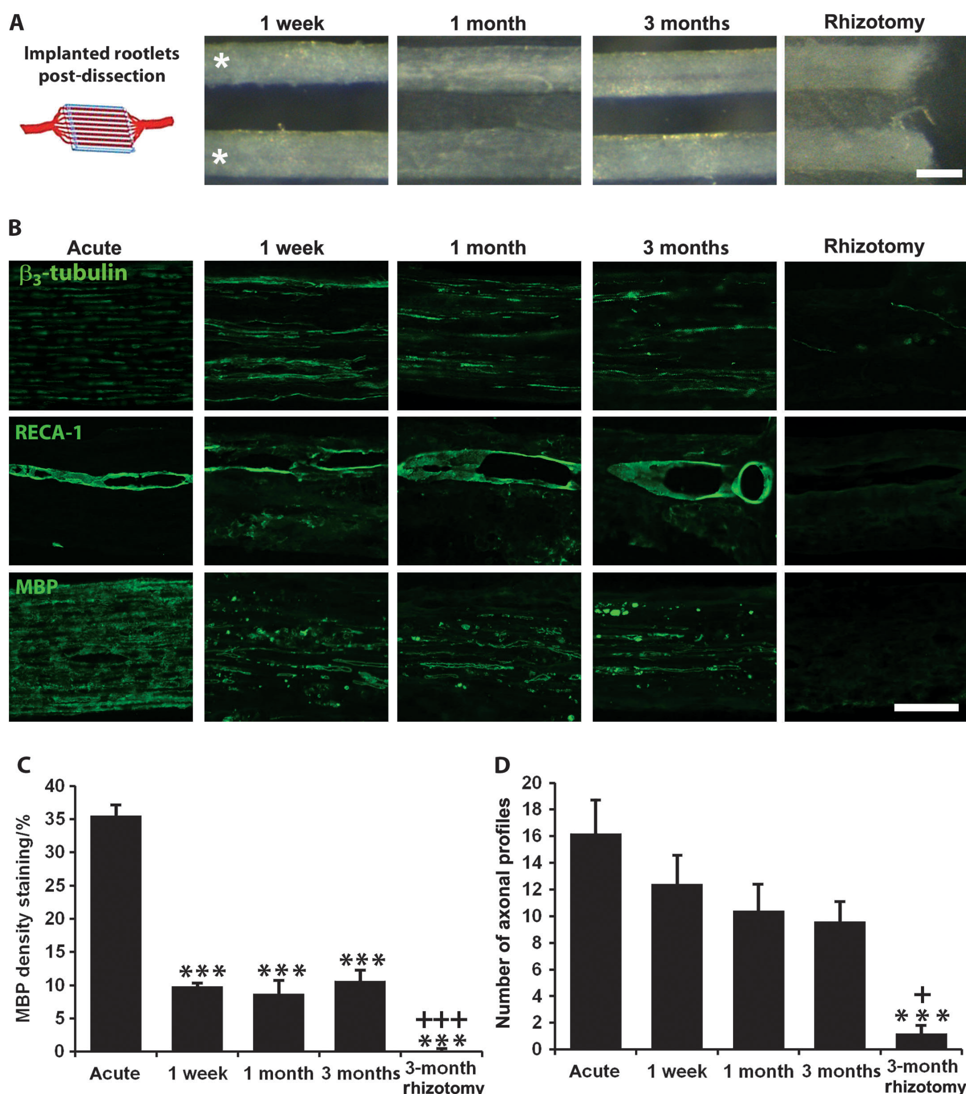

Fig. 3. Axon count, blood vessel survival, and myelination of implanted dorsal rootlets. (**A**) Chronically implanted rootlets (white asterisk) were removed from microchannels and cryosectioned. Scale bar, 100 μm. (**B**) Longitudinal sections of non-rhizotomized implanted rootlets immediately after teasing (acute), at 1 week, 1 month, 3 months, and 3 months with rhizotomy. Expression of the antigen markers for axons (β_3-tubulin), blood vessels (RECA-1), and myelin (MBP) was detected by immunohistochemistry. Complete loss of staining is clear in the rhizotomy group. Scale bar, 50 μm. (**C**) Mean percentage density of MBP in implanted rootlets immediately after surgery (acute) and for 1 week to 3 months including rhizotomy. (**D**) Mean number of β_3-tubulin–positive axons in sections of implanted rootlets immediately after surgery (acute) and from 1 week to 3 months including rhizotomy. $^{***/+++}P < 0.001$, $^{+}P < 0.05$, analysis of variance (ANOVA) plus Bonferroni post hoc ($n = 4$ per group). Error bars are SEM. "*" refers to comparison with acute, and "+" refers to comparison of non-rhizotomized to rhizotomized at 3 months.

driven by spinal neural input (*36*). Cutaneous stimulation, either electrically or manually, produced activity in all rootlets. These experiments demonstrate that the information required for bladder control is accessible from teased rootlets. The high-amplitude firing associated with bladder emptying is readily distinguishable and could be used to trigger a SARS device in patients with spinal cord injury to stop unsolicited voiding. Smaller amplitude waveforms can provide reliable information about bladder fullness.

Spike sorting analysis. Distinct action potentials could be recorded from the rootlets in response to cutaneous stimulation, bladder filling, and bladder contraction (when infused beyond 0.4 ml); some units fired continually and unsolicited. During bladder infusion at volumes below the threshold for reflex micturition, multiunit activity was recorded (Fig. 5, A and B) and many individual waveforms were distinguishable through digital spike sorting; in this example, three were distinguished (Fig. 5C). Through offline analysis, these three individual waveforms are converted into a firing frequency scatter plot across time (Fig. 5D). Data from each waveform histogram were subjected to correlation analysis against bladder infusion volume. High R^2 values indicated high correlation between firing rate and bladder fullness in these three example waveforms (Fig. 5E).

Active microchannels. Microchannels containing tripolar gold recording electrodes were tested for comparative performance against conventional hook electrodes. Teased rootlets were confined within five tripolar electrode-active microchannels, connected with the guard electrodes at the ends of the channels as reference. The muscle layers were sutured closed, and wires were externalized for recording (Fig. 6, A and B). The peak-to-peak noise level for silver hooks in oil averaged 22 μV, whereas that for microchannels in saline was 17 μV: the amplitudes of spikes from cutaneous stimulation and

correspond to normal rat bladder physiological volumes. When infusion was continued to a physiologically full volume (~0.5 ml), spikes of much larger amplitude and firing frequency were elicited, corresponding with a sharp rise in bladder pressure in the range of 30 to 50 cm H_2O, indicating reflex bladder contraction (*34*). These spikes probably correspond to muscle spindle afferents innervating the pelvic floor and urethral sphincter such as the pubocaudalis and rhabosphincter (*35*). Small fluctuations in pressure could be seen throughout the infusion period; these are spontaneous nonmicturition contractions that are not

bladder filling were comparable (fig. S3). This excellent signal quality, even in saline, reflects tripolar noise reduction and signal amplification from microchannel confinement. Bladder pressure was measured alongside the five concurrent recordings of neural activity from the channels, an example of which is illustrated (Fig. 6E). Using spike processing, it was possible to identify multiple waveforms in each microchannel, several of which had a firing frequency correlated with bladder filling (Fig. 6F). This is illustrated in channels 2 and 3 because they show no activity before filling and then a "ramping" increase during infusion. The activity of waveform 3b

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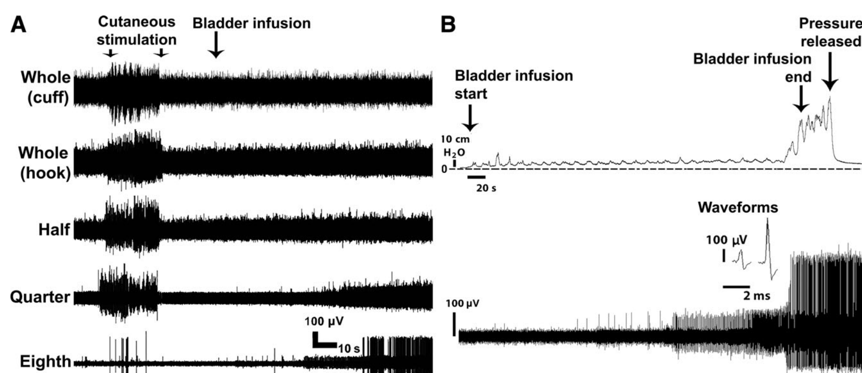

Fig. 4. Distinguishable waveforms are observed during pump infusion of the bladder, but only after surgical reduction of rootlet diameter. (**A**) Recording from the whole L6 dorsal root using a nerve cuff in saline and hook-in-oil preparations. After sequential surgical teasing (half, quarter, and eighth of the diameter), afferent response to bladder infusion was detected. (**B**) Typical recording from an acutely teased rootlet in response to saline infusion. The sudden rise in pressure at 6 min indicates micturition. There are two distinct waveforms that are initiated at different levels of bladder pressure/volume (inset). The smaller amplitude waveform encodes bladder filling, and the larger amplitude waveform encodes bladder contraction/micturition. Infusion ends when the catheter/transducer is removed, and the animal is allowed to empty its bladder. Note that afferent activity and pressure remain high, beyond the end of infusion, because of the continued contractile phase. cm H_2O, bladder hydrostatic pressure.

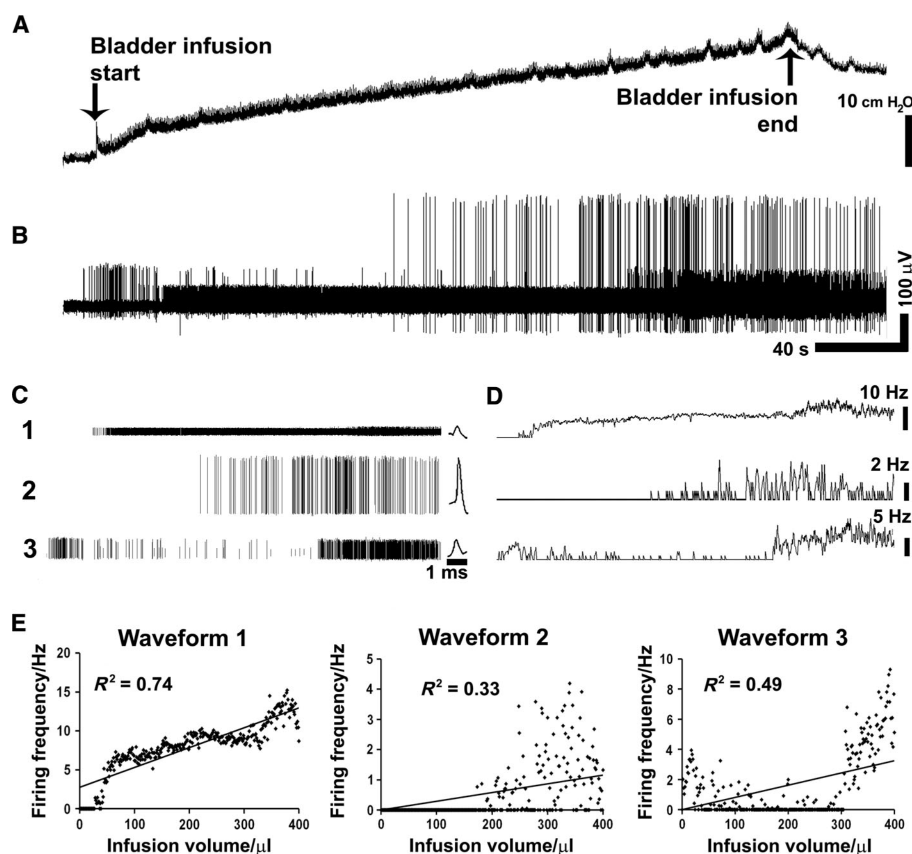

Fig. 5. Spike sorting and bladder volume firing frequency correlation analysis. (**A**) Raw data of bladder pressure during infusion. (**B**) Afferent activity recorded from one representative implanted dorsal rootlet. (**C**) Upon spike sorting, three waveforms with different amplitude and firing rate can be distinguished in response to bladder filling. (**D**) These waveforms are converted to a firing frequency histogram. (**E**) Firing rate against volume infused plotted, and analysis determining the correlation coefficient (R^2) of related activity, showing R^2 for waveforms 1 (0.74), 2 (0.33), and 3 (0.49).

especially, indicated by the black asterisk in Fig. 6F, could be correlated accurately with bladder pressure (R^2 = 0.76; Fig. 6, G and H). Action potential SNRs of waveforms at implantation were excellent across all five channels, with the largest SNR > 17:1 and peak-to-peak amplitudes of 410 μV. Some rootlets (channels 4 and 5) also had unit activity uncorrelated to bladder filling or cutaneous stimulation, likely encoding postural proprioceptive afferents from the sacrum. In all channels, dermatome stimulation elicited spike activity. Because channels contained axons carrying various forms of information, and because only half of the rootlets/channels carried bladder fullness information, multichannel concurrent recordings will be necessary to be assured of recording bladder waveforms. These results show that bladder sensory information can be recorded using microchannels suitable for chronic implantation, equivalent in quality to that obtainable with the optimal acute setup, that is, hooks under oil.

Chronic recordings from teased dorsal roots in microchannels

Having established the baseline electrophysiological function of acutely dissected rootlets, we compared this to the activity of rootlets implanted in microchannels and left in place for up to 3 months.

Hook electrode. Spike activity was recorded from rootlets acutely, at 1 week, 1 month, and 3 months after implantation, with representative rootlet recordings and corresponding waveforms shown in Fig. 7A. Spike activity was elicited in response to cutaneous stimulation and from progressive bladder filling. All rootlets conveyed at least one cutaneous waveform (Fig. 7B). For the implanted non-rhizotomized rootlets, cutaneous activity could be recorded from 91 ± 5.6% of rootlets at 1 week, 89.7 ± 4.5% at 1 month, and 94.3 ± 3.5% at 3 months after implantation (Fig. 7B). In comparison, in the rhizotomy group, only 64.3 ± 12.0% of implanted rootlets could be stimulated cutaneously at 3 months, and often, only a single unit could be detected (Fig. 7B). This was significantly less than both acute [$t(10) = 3.38$, $P < 0.01$] and 3-month non-rhizotomized [$t(10) = 2.66$, $P < 0.05$] groups, suggesting that most axons in these rhizotomized implanted rootlets had died, corroborating the histology.

Recording of neural activity in non-rhizotomized rootlets in response to bladder

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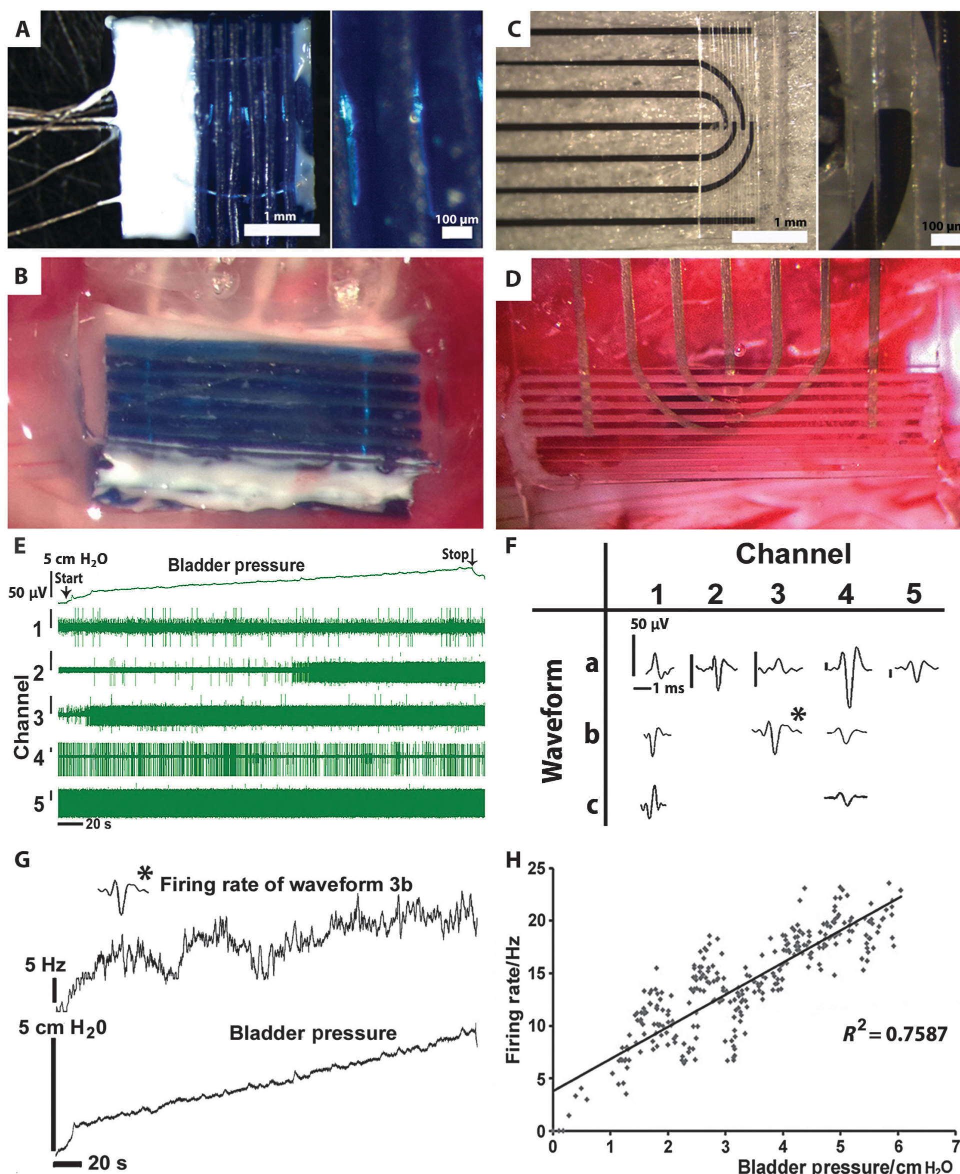

Fig. 6. Concurrent recording of teased L6 dorsal rootlets implanted within the microchannel neuroprosthesis during bladder infusion. (**A** to **D**) Prototype (A and B) and microfabricated microchannel electrodes (C and D) are implanted intrathecally around the teased L6 dorsal rootlets of rats with channels parallel with the vertebral axis. (**E**) Neural recording from device during artificial bladder filling (top trace) in all five channels (traces for channels 1 to 5). (**F**) Multiple waveforms sorted from each implanted rootlet. Some channels have multiple waveforms that can be discriminated. (**G** and **H**) Trace of firing frequency of waveform 3b [asterisk in (F)] with respect to bladder pressure (G) and subsequent correlation analysis (H). Afferents such as these can give a reliable indication of bladder filling; however, many other afferents do not show such correlative activity. $n = 4$ replicates.

high-frequency firing occurred during bladder contractions. This demonstrates that about 90% of rootlets have functional axons at 3 months after microchannel implantation with no significant loss of bladder specificity. However, rhizotomized implanted rootlets retained very few functional bladder afferents, confirming histological results.

Active microchannels. In the confined space of the rat pelvis and hindquarters, long-term implantation of microchannels with connectors led to implant movement and rootlet damage. To examine long-term recording from implanted active microchannels, we performed prolonged recording under anesthesia. Cutaneous electrical stimulation of the L6 dermatome over 12 hours revealed a common pattern of compound activity within the microchannels (fig. S4). Acutely at implantation, peak-to-peak amplitudes of more than 200 μV and average conduction velocities ranging from 25.5 to 1.56 m/s could be seen. The velocities of the peak spike were 21.5 ± 1.25 m/s, suggesting that Aα-β axons predominate in the compound action potential, with slower unit activity falling within the range of thinly myelinated Aδ and C axons, in accordance with published literature on primary afferent classification (*37*, *38*). The slowly conducting subtypes are typically those that innervate the bladder (*33*). Over the course of 12 hours, conduction velocities showed a slight but not significant reduction ($F_{4,15} = 1.178$, $P > 0.05$). At 3 hours, peak spike velocity was 10.4 ± 3.39 m/s, but this recovered at 6 hours (15.4 ± 2.68 m/s) and remained constant for up to 12 hours (15.1 ± 2.8 m/s). This is the same value that we obtained for conduction velocities at 3-month implantation in a separate rat. Afferent waveforms that correlated to bladder infusion could be detected across the 12-hour implantation; three such waveforms are indicated (fig. S4). A slight amplitude diminishment was seen in waveforms 1 (106 to 65 μV) and 3 (52 to 20 μV), but not in waveform 2, including the cutaneous afferents responding to dermatomal stimulation (210 to 235 μV).

filling (Fig. 7C) after 3 months of implantation showed that 42.9 ± 8.13% of rootlets carried at least one correlated bladder waveform (similar to acute recordings, 54.9%). However, in the rhizotomy implantation group, only 11.3 ± 7.0% of rootlets carried detectable bladder filling information, significantly lower than naïve [$t(10) = 2.26$, $P < 0.05$] and 3-month nonrhizotomized [$t(10) = 2.85$, $P < 0.05$] rootlets. In the nonrhizotomized group, similar to acutely teased dorsal roots, large-amplitude

Again, amplitudes are comparable to those recorded at 3 months. The small reduction in either amplitude or conduction velocity over this period could be due to partial anoxia or surgical damage, and this correlates with the loss of myelin (complete at 1 week). These results show that implantation of teased rootlets in microchannels, over the initial 12 hours when anoxia might be at its worst, does not cause further damage sufficient to affect axonal conduction.

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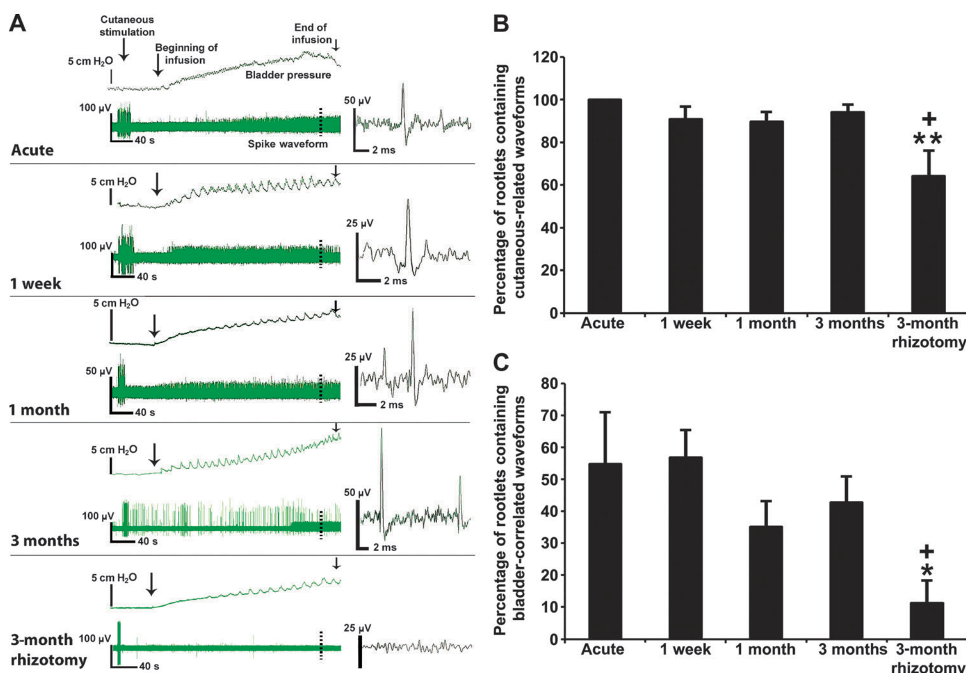

Fig. 7. Recorded waveforms of acute and chronically implanted rootlets during cutaneous stimulation and bladder filling. (**A**) Representative afferent activity recorded from an implanted non-rhizotomized rootlet in acute, 1 week, 1 month, 3 months, and 3-month rhizotomized animals during cutaneous stimulation and bladder infusion (top trace). Spikes shown at the beginning of the trace are in response to cutaneous stimulation. The dotted line in the raw spike trace indicates the point where the waveform to the right of the trace refers. (**B**) Percentage of implanted L6 rootlets responding to cutaneous activity immediately after surgery (acute) and for 1 week, 1 month, and 3 months, including rhizotomy. (**C**) Mean percentage of rootlets that contain at least one correlated bladder afferent acutely and for 1 week, 1 month, and 3 months, including rhizotomy. $^{**/++}P < 0.005$, $^{*/+}P < 0.05$, Student's t test ($n = 5$ per group). Error bars are SEM.

Prevention of bladder emptying using conduction block

For a useful bladder neuroprosthesis, fullness or voiding information must be acquired and then used to prevent unsolicited bladder contractions. We investigated one way of aborting bladder emptying by blocking motor axon conduction to the detrusor muscle, by applying a high-frequency depolarizing blockade. Low-frequency stimulation of the ventral roots at 30 Hz caused an immediate increase in bladder pressure, but these contractions could be completely prevented by application of a 20-kHz depolarizing block (Fig. 8A). The block remained effective for about 20 s after cessation, after which low-frequency stimulus again elicited bladder contraction. In humans with full bladders, spontaneous voiding contractions occur every few minutes, whereas in rats with a full bladder, we saw regular contractions about every 10 to 20 s, in accordance with previous data (*34*). These spontaneous bladder contractions initiated by bladder filling were also prevented by a depolarizing block. Again, contractions resumed about 40 s after cessation of the block (Fig. 8B). Repetitive blocking had no detrimental effect.

DISCUSSION

We describe a method suitable for long-term recording from bladder sensory afferents in rat. This was achieved by microdissection of dorsal roots into rootlets, which were placed into microchannels. Using spike processing, we accomplished robust single-unit recordings conveying information on bladder fullness and contraction. Microchannel recording provides a significantly improved SNR compared to whole-nerve cuff recording, comparable in amplitude and SNR to acute hook electrode recordings under insulating oil. A method to abort bladder emptying using a motor nerve conduction blockade was also demonstrated. Together, these findings put in place the necessary tools to attempt a revision of the bladder control device initiated by Brindley more than 30 years ago (*5*). The notable advance from the perspective of patients is that the modified device would be closed-loop and could work without the necessity of cutting the dorsal roots from the spinal cord.

The key to the development of an automatic bladder control strategy is the ability to record bladder-related sensory information. Previously, this has been challenging because of the relatively small extracellular potentials generated. A previous attempt at recording bladder fullness information from a human patient intraoperatively using an electrode cuff demonstrated potentials of less than 5 μV (*21*). Tripolar microchannel electrodes suppress noise and increase the effective extracellular impedance in the vicinity of the electrodes, thereby amplifying the extracellular potential to hundreds of microvolts (*28*, *39*). This has enabled the identification of specific sensory modalities of reflex bladder emptying and fullness from a mixed nerve root, which receives divergent sensory information from the skin, viscera, musculature, and reproductive system (*40*, *41*). Microdissection of these dorsal roots is essential to improve SNR and enable implantation into microchannels. Here, we present a surgical technique for teasing rat dorsal rootlets with optimal long-term survival. Some damage occurred in the rootlets during this surgery, leading to partial demyelination, but no significant axon loss. After this initial surgical trauma, the rootlets continued to conduct action potentials for at least 3 months, with about half conveying information on bladder fullness. All channels contained cutaneous sensory axons, so multichannel devices will be required for reliable recording of bladder fullness.

The current study confirms our previous studies reporting that rootlets acutely confined within microchannel electrodes produce excellent SNR (*30*) and that a proportion carry bladder information (*31*). Only non-rhizotomized rootlets retained long-term function. Rhizotomy led to axon, myelin, and blood vessel loss and poor bladder afferent recording. Maintaining dorsal root continuity with the spinal cord will be essential in a revised bladder neuroprosthesis.

Whereas obtaining information on bladder fullness requires spike processing, detection of the onset of voiding is easier. High-frequency/high-amplitude firing of muscle spindle afferents accompanies bladder

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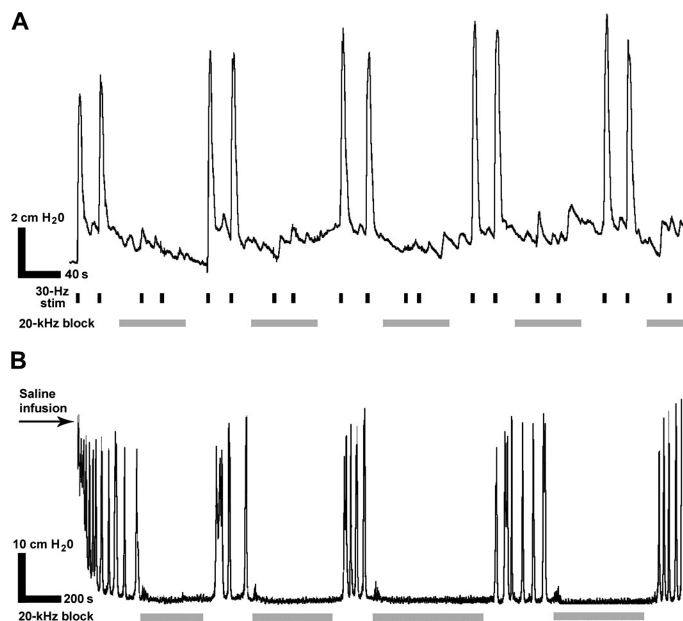

Fig. 8. Electrical stimulation– and infusion-induced bladder contraction inhibited by high-frequency conduction block. The bladder smooth muscle can be stimulated to either contract or relax depending on the frequency of alternating current. (**A**) Stimulation (30 Hz) of the bilateral L6 ventral roots produced contractions of the bladder (horizontal black bars). Preemptive 20-kHz stimulation from tungsten foil cuff electrodes prevented electrical stimulation–induced bladder contraction (gray bars). (**B**) Natural bladder contractions, elicited by bolus saline infusion into the bladder, could also be inhibited by high-frequency stimulation (gray bars). In both cases, the block was reversible after 20 to 40 s of recovery. $n = 3$ replicates.

contraction. Having detected voiding onset, a high-frequency stimulus conduction block at the level of the ventral root could be used to prevent bladder emptying. This is an effective way to block conduction in the sciatic nerve (*42*) as well as ventral roots (*43*) and pudendal nerves (*44*) that innervate the urethral sphincter. The latter technique enables micturition through an open sphincter, avoiding DSD, and has been effectively shown in a human patient (*45*). In our experiments, we used a depolarizing block to prevent artificial and natural bladder contractions. There are alternative methods of reflex micturition inhibition, which could also be effective. These include low-frequency collision block of the dorsal roots (*23*) and pelvic afferents (*46*) as shown in animal models. Noninvasive penile nerve stimulation has worked particularly well at suppressing bladder contractions in patients with spinal cord injury (*47*, *48*), and the same benefit has been shown when SARS has been applied to extradural mixed or intradural posterior sacral roots (*49*). The hypothesized mechanism for this particular "neuromodulation" is afferent-directed inhibition of detrusor muscle motor neurons (*50*). However, high-frequency conduction block has advantages. It inhibits bladder detrusor muscle activity entirely and immediately, as would be needed to abort a reflex bladder contraction. Additionally, because stimulating electrodes are already implanted around the ventral roots in the Finetech-Brindley system, no further invasive surgery or cumbersome external stimulation systems need to be applied. Modified SARS electrodes could be used to drive a high-frequency block of the ventral roots, although the same electrodes stimulated at low frequency would drive bladder emptying when required. The main disadvantage of high-frequency blockade may be power consumption.

DSD associated with hyperreflexia is clearly a problem after spinal cord injury, leading to high bladder pressures during micturition and kidney damage, the prevention of which is a primary urological goal in patient management (*51*). Anodal collision block of the ventral roots has been shown to differentially inhibit large efferents of the sphincter muscles while bypassing the smaller efferents innervating the detrusor smooth muscle (*52*–*54*). This stimulation paradigm, or a depolarizing block of the pudendal nerve, could produce coordinated detrusor-sphincter activity during device-induced micturition.

The development of the microchannel recording interface, together with surgical preparation of dorsal rootlets avoiding rhizotomy, accompanied with high-frequency block, opens possibilities for redesign of the SARS stimulator. The sensory recording of afferent activity will provide an indication of when the bladder is full and when it undergoes spontaneous contraction. This information could be used to drive a direct high-frequency conduction block of the bladder motor axons to prevent spontaneous emptying. Bladder fullness may be determined by a frequency threshold of afferent activity in a subcutaneously dwelling "fullness monitor," currently under development (*55*). When bladder emptying is required, low-frequency ventral root stimulation could be accompanied with anodal block of the external sphincter efferents, providing synergic micturition in a fully integrated bidirectional neuroprosthesis.

MATERIALS AND METHODS

Study design

We have conducted a feasibility study in rats into the surgical implantation of microchannel electrodes onto dorsal roots, accompanied with neuromodulation to ventral roots, for determining the potential of a closed-loop neuroprosthetic treatment for neurogenic bladder.

Animals

Sprague-Dawley rats ($n = 52$, female, 250 to 300 g, Harlan Laboratories) were used in the experiments. Forty-five underwent surgical implantation of teased L6 dorsal rootlets into PDMS passive microchannel devices (Supplementary Materials and Methods). In most animals, dorsal rootlets were kept in continuity with the spinal cord and were assessed acutely, at 1 week, 1 month, and 3 months. A 3-month implant group combined with proximal rhizotomy was used as a comparison. Twenty animals were used for histological and ultrasound analysis ($n = 4$ per group), and 25 were used exclusively for electrophysiological recording ($n = 5$ per group). In addition, three animals underwent high-frequency conduction block on bladder ventral roots, during bladder stimulation with either saline filling or low-frequency electrical current. Finally, four animals were used to assess the functional capabilities of the active microchannel devices (see the Supplementary Materials) across a 12-hour time course study. All experimental procedures were performed in accordance with the UK Animals (Surgical Procedures) Act 1996.

Dorsal root surgery

Animals anesthetized with isoflurane [2% in O_2 (2 liters/min)] received laminectomies of L1-L3 vertebrae. The L6 dorsal root was "teased"

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apart with a pair of fine tip glass probes into five to nine rootlets of diameter ~100 μm. Rootlets were laid into 3-mm-long microchannels, a PDMS lid was attached, and the implant was padded with gel foam. In one group, implantation was also accompanied with rhizotomy at the DREZ. The animals assessed had an average of six (acute), six (1 week), seven (1 month), six (3 months), and five (3 months rhizotomized) rootlets implanted. Implantation of electrode-active microchannels was carried out in the same way, and in addition, recording wires with connector pins remained in situ subcutaneously.

Neural activity recordings using passive microchannel implants

Electrophysiological recording (see the Supplementary Materials) of rootlets within PDMS microchannels was performed terminally. Microchannel implants were recorded initially by penetrating with tungsten electrodes (impedance 1 to 2 megohms at 1 kHz) (*39*). However, the high impedance and substantial rootlet damage sustained from penetration resulted in poor SNR (fig. S1). Accordingly, we lifted rootlets from microchannels and recorded with silver hook electrodes under oil (impedance <100 kilohms) as traditionally described (*56*). Epidermal electrical stimulation (3 mA, 3 ms, 20 Hz) was applied via a pulse generator, buffer, and stimulus isolator (Digitimer) to the L6 dermatome at 3 months to determine afferent conduction velocities after chronic implantation compared to acute.

Neural activity recordings using active microchannel implants

Electrophysiological assessments were also performed using electrode-active microchannels, avoiding relaminectomy and implant reexposure. Active microchannels were implanted into four rats, anesthetized with urethane, encapsulating five teased rootlet strands of the L6 dorsal root. The animals were sutured in layers, with the enameled silver wires exposed through overlying muscle and skin, and the bladder was catheterized. The five microchannels were recorded concurrently during bladder infusion initially at implantation, and then at 3, 6, 9, and 12 hours. Manual and electrical epidermal stimulation were also applied.

Bladder contraction induction and inhibition by conduction block

Separate experiments were conducted to establish the efficacy of high-frequency alternating current conduction block on micturition. In three female rats, the lumbar ventral roots were exposed via a laminectomy of L1-L4 vertebrae. The bilateral L6 ventral roots were placed within a tungsten foil electrode cuff (*43*). Electrodes were connected via two constant current stimulators, each by a series 1-μF nonpolarized capacitor and 4.7-kilohm resistors, to a biphasic buffer (Digitimer) and a Power 1401 (Cambridge Electronic Design) controlled by Spike2 software (Cambridge Electronic Design). Sinusoidal waveforms at 20 kHz and 7.5 V were delivered. To initiate contractions, we stimulated the ventral roots (30 Hz, 1 ms, 20 μA) with silver hook electrodes proximal to the cuff. Alternatively, the bladder was stimulated naturally by an infused saline bolus (0.5 ml) through a one-way catheter, preventing expression.

Bladder volume measurements

An ultrasound scanner (MicroMaxx, Fujifilm SonoSite) was used to measure residual urine volume before electrophysiology assessment of microchannel implantation in the rhizotomized group. After sacrifice, the emptied bladders were removed and measured.

Data analysis

Spike2 software performed action potential waveform sorting from the raw channel recordings. Waveform parameters were kept consistent: a minimum of 50 spikes were required for a positive waveform description. High- and low-level thresholds removed false positives and noise contaminants. Waveforms were converted to a firing frequency, sampled at 100 Hz.

Tissue preparation and immunohistochemistry

Animals received intraperitoneal pentobarbitone and were transcardially perfused with paraformaldehyde (PFA) (4% in 0.01 M phosphate buffer, pH 7.4). Rootlets were dissected, postfixed in PFA for 2 hours, and stored in 20% sucrose. Sections (8 μm) were cut and mounted on Superfrost Plus slides (VWR). Slides were washed twice for 10 min each in 0.01 M phosphate-buffered saline (PBS) and incubated for 1 hour at room temperature in 10% normal donkey serum (NDS) with 2% bovine serum albumin (diluted in 0.01 M PBS), followed by β_3-tubulin (rabbit, 1:1000), RECA-1 (mouse, 1:50), or MBP (mouse, 1:1000) (all antibodies diluted in 10% NDS in 0.01 M PBS and obtained from Abcam) for 24 hours. After washing the slides, anti-rabbit or anti-mouse Alexa Fluor 488 (donkey, 1:1000 diluted in 0.01 M PBS, Invitrogen) was applied for 2 hours. Slides were washed and coverslipped with FluorSave (Merck).

Microscopy

Sections at ×20 magnification (three per animal) were examined for quantitation of β_3-tubulin–positive axons crossing an arbitrary centerline of the rootlet section. Sections were analyzed for percentage density staining of MBP in a selected 100 μm^2 of rootlet through ImageJ software, after a consistent binary intensity threshold.

Statistical analysis

Significant differences are expressed as means ± SEM. Statistical comparisons between immunohistochemistry groups were conducted with a one-way ANOVA and Bonferroni post hoc analysis. Ultrasound measurements and electrophysiological groups are compared with Student's t test. F and T values, degrees of freedom [between groups, within groups], and P values are shown in the text.

SUPPLEMENTARY MATERIALS

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Material and Methods
Fig. S1. Tungsten needle and platinum hook electrode recording from implanted dorsal rootlets during electrical stimulation of the L6 dermatome.
Fig. S2. Neurogenic complications are not evident in the bladder after surgical manipulation and implantation of the L6 dorsal roots into microchannels.
Fig. S3. Comparison of microchannel (in saline) versus hook electrodes (in oil).
Fig. S4. Afferent waveforms recorded from active microchannels across 12 hours, responding to dermatome electrical stimulation and bladder infusion.

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