Received: 23 March 2017 | Accepted: 27 December 2017
DOI: 10.1002/nau.23504

ORIGINAL BASIC SCIENCE ARTICLE

WILEY Neurourology Urodynamics ICS SUFU

The frequency spectrum of bladder non-voiding activity as a trigger-event for conditional stimulation: Closed-loop inhibition of bladder contractions in rats

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Aims: To test the hypothesis that the frequency of bladder non-voiding contractions (NVCs) can be used as a trigger event for closed-loop conditional inhibition of detrusor contractions via tibial nerve (TN) or dorsal penile nerve (DPN) stimulation.
Methods: In urethane anaesthetized male Wistar rats, the bladder was filled continuously with saline to evoke contractions. To test the plausibility of conditional inhibition via the TN, electrical stimulation was switched on manually when the pressure increased above a threshold of 10 cmH_20 above the baseline. For testing conditional stimulation via the DPN, the pressure signal was continuously stored and a baseline threshold, the area under the curve (AUC) of the amplitude spectrum in the 0.2–20 Hz range of a 5 s window at the beginning of filling was calculated. When the AUC of subsequent pressure windows superseded the baseline threshold, the DPN was automatically stimulated.
Results: TN stimulation failed to inhibit evoked voiding contractions. The NVC frequency spectrum based DPN stimulation successfully inhibited 70% of the evoked contractions and resulted in a 45% increase in bladder capacity (BC).
Conclusions: While, conditional TN stimulation failed to suppress bladder contractions, DPN stimulation, automatically triggered by an increased frequency of bladder non-voiding activity, resulted in bladder inhibition, and a consequential increase in BC. This study demonstrates the plausibility of using the frequency of NVCs as a trigger event for conditional inhibition of detrusor contractions.

KEYWORDS
conditional stimulation, neurogenic detrusor overactivity, neuromodulation

1 | INTRODUCTION

Spinal cord injury may cause disruption of the coordinated neural signaling between the central nervous system and the bladder. The lack of regulatory input from higher centers often results in involuntary contractions of the bladder muscle in the storage phase.[1] This condition, neurogenic detrusor overactivity (NDO), is

Karl-Erik Andersson led the peer-review process as the Associate Editor responsible for the paper.

symptomized by urinary incontinence, urgency, and frequency.[2] Neuromodulation is an important treatment for NDO patients who are refractory to pharmacologic interventions.[3]

Stimulation can be applied either continuous[4] or conditional.[5] Continuous stimulation has been found effective in suppressing detrusor contractions and in restoration of bladder capacity (BC).[6] However, longer stimulation periods might result in ineffectiveness due to adaptation, increased risk of tissue damage, and high battery consumption.[5] In contrast, conditional stimulation is triggered only when an impending bladder contraction is detected[5,7] and may therefore considerably reduce the stimulation duration and thus, the above-stated complications. A number of contraction detection methods such as electromyography of the bladder,[8] the urethral,[9] and the anal sphincter[10] as well as electroneurography of the sacral root,[11] the pelvic nerve,[12] and the pudendal nerve[13] have been described. However, due to difficulties in signal acquisition and low signal-to-noise ratio, none of these methods have been used in clinical practice. The most commonly used method triggers stimulation when a preset bladder pressure (10–15 cmH_2O) is exceeded.[5,7,14] Although these methods have been found useful in suppressing bladder contractions, they fail to alert patients of an impending voiding perceived as "urgency," which has been reported by patients about 5 s before a bladder pressure rise of 10 cmH_2O.[7]

In pursuit of developing a method for conditional neurostimulation triggered by urgency, we recently reported that voiding in rats is preceded by recurrent changes in frequency of bladder non-voiding activity.[15] To detect these changes, a moving average, Fast-Fourier Transform algorithm was developed, and validated offline on pre-recorded pressure measurements.

In our current study, we aimed at expanding the developed algorithm to real-time, to show that the frequency of non-voiding contractions (NVCs) can be used as a trigger for closed-loop conditional inhibition of detrusor contractions. To this end, we tested the plausibility of conditional stimulation of the tibial nerve (TN), which has mostly been used for continuous stimulation.[4] We also tested our technique with the conditional stimulation of the dorsal penile nerve (DPN).

2 | METHODS

2.1 | Animals

The local Animal Care and Use Committee approved experiments described in this study. Male Wistar rats ($n = 18$, 440 ± 24g) were used.

2.2 | Bladder filling and pressure measurements

The animals were anesthetized by intraperitoneal administration of urethane (50% g/v, 1.2 g/kg body weight). A midline incision was made to expose the abdominal cavity. Bladder pressure measurement and bladder filling (0.06 mL/min) were done through a 23 G, needle inserted at the top of the bladder. The needle was connected to a disposable pressure transducer and an infusion pump using a 3-way connector. Pressure was measured using a Statham SP1400 blood pressure monitor and was displayed in real-time on a computer screen using a custom written LabVIEW® program. The pressure was sampled at 25 Hz and was analyzed with a custom written MATLAB® program.

2.3 | Types of experiments

2.3.1 | Conditional tibial nerve stimulation

The TN was accessed via the medial side of the right hind leg near the ankle. For stimulation a bipolar cuff electrode was placed around the nerve. The bladder was filled continuously and 2-3 control cystometrograms (CMGs) were recorded. Then the TN was stimulated with monophasic rectangular pulses of frequency 5 Hz, width 200 or 400 μs and with an initial amplitude approximately three times the motor threshold (T) to induce a slight toe movement. The amplitude was increased in steps of 1 V until an inhibition of the voiding reflex or a maximum amplitude of 5 T was reached. The stimulation was manually switched on/off when the pressure

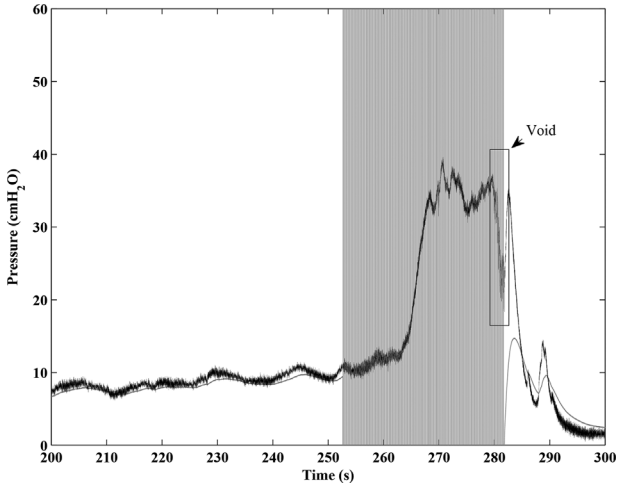

FIGURE 1 An example of unsuccessful conditional stimulation of the tibial nerve. The stimulation was manually switched on when the bladder pressure increased 10 cmH_2O above the baseline. The shaded region shows the stimulation period, where it is noticeable that regardless the stimulation, the pressure increased rapidly, ultimately culminating to a voiding

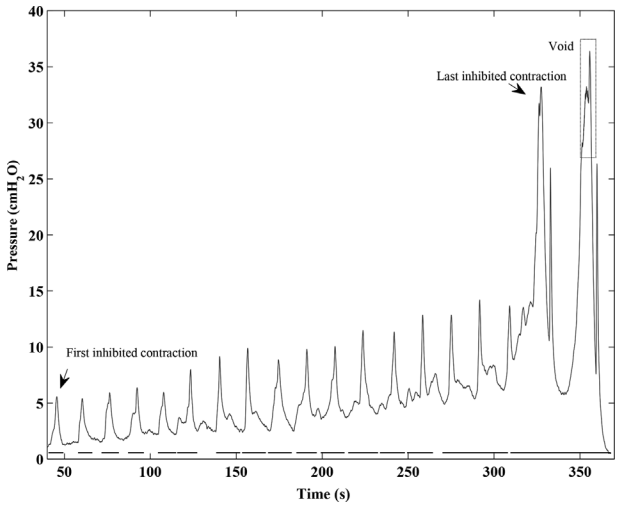

FIGURE 2 An example of conditional stimulation of the DPN. The stimulation was automatically switched on when the area under the curve of the frequency spectrum of pressure signal superseded the predefined threshold. As seen in the figure, a series of contractions were evoked and subsequently suppressed. The contractions could only be inhibited up to a certain volume, thereafter voiding was observed. The horizontal bars represent the stimulation period

increased above a threshold of 10 cmH_20 above the baseline, or decreased below the threshold.

Urodynamic parameters

Voiding in rats is marked by rapid contractions of the urethral sphincter, seen as high frequency oscillations (HFOs) in bladder pressure recordings. We calculated the maximum pressure (p_{max}) immediately before the start of HFOs, the baseline pressure (p_{basal}) as the mean of a 5 s pressure window at the beginning of filling and the BC as the volume required to evoke a voiding (Figure 3). We also calculated the rise time from p_{basal} to p_{max} and the decay constant which gives a measure of the rate of exponential pressure decay after a voiding[16] (Figure 1).

2.3.2 | Conditional dorsal penile nerve stimulation

A bipolar platinum-iridium electrode was mounted on a branch of the DPN. According to the described anatomy of the sensory branch of the pudendal nerve in the male rat, we stimulated the so called middle branch.[17] Plastic tubing was wrapped around the nerve-electrode system to isolate it from the surrounding tissue. The bladder was filled continuously and 2-3 control CMGs were recorded. Stimulation amplitude was determined during the control CMGs by stimulating the DPN, initially with an amplitude of 1 V, and subsequently increasing it until a fall in bladder pressure was observed. A computer program for real-time frequency domain analysis based stimulation was written in LabVIEW®. It calculated the area under the curve (AUC) of the pressure amplitude spectrum in the frequency range 0.2-20 Hz. From the control measurements a baseline threshold, the AUC of the pressure signal at the beginning of filling, and a stimulation threshold, the AUC of a 5 s pressure window just before a voiding contraction were calculated. When, during the subsequent filling cycles, the AUC value of a 5 s pressure window superseded the stimulation threshold, a pulse train of frequency 10 Hz, width 200 μs, and a pre-defined amplitude was automatically switched on to stimulate the DPN. The stimulation stopped again automatically when the value of AUC fell to the baseline threshold.

Urodynamic parameters

As the filling progressed, bladder contractions were evoked and subsequently inhibited until voiding (or leakage) occurred. For each of these stimulation CMGs, we calculated the p_{max} at the first and the last inhibited contraction (Figure 2). We also calculated the p_{max} at the beginning of a voiding contraction in control and stimulation CMGs. For stimulation measurements, the change in the BC was defined as the extension of the filling time from the first inhibited

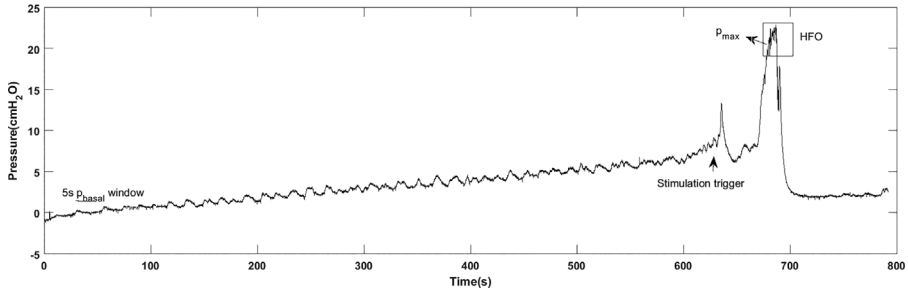

FIGURE 3 Voiding in rats is marked by rapid contractions of the urethral sphincter, seen as high frequency oscillations (HFOs) in bladder pressure recordings. The maximum pressure (p_{max}) represents the value immediately before the start of HFOs, the baseline pressure (p_{basal}) was calculated as the mean of a 5 s pressure window at the beginning of filling. This is an example of an "On time" stimulation

contraction up to the occurrence of voiding. The rationale behind this is that if the bladder was not stimulated, voiding would have occurred at the first inhibited contraction.[5] We also calculated the ratio $p_{basal\ (after\ stimulation/before\ stimulation)}$. The automatic stimulation was considered "on time" if the change in AUC threshold (hence the start of stimulation) occurred at the onset of a visual progressive rise in pressure above the baseline. If the stimulation did not start at the onset of pressure rise it was considered "late".

2.4 | Statistical analysis

All data are presented as mean ± SD. A Shapiro-Wilk test was done for normality testing, and Levene's test for homogeneity of variance. To compare groups, an ANOVA followed by Bonferroni test for multiple comparisons was carried out on normally distributed data, and Kruskal-Wallis test on non-normal data using the SPSS® statistical package (version 21.0, SPSS Inc., Chicago, IL). $P < 0.05$ was considered significant.

3 | RESULTS

3.1 | Conditional tibial nerve stimulation

The average motor threshold, T, to induce a slight toe movement was 1.42 ± 0.4 V. Manual conditional stimulation of the TN with amplitude 3-5T failed to inhibit any of the 50 voiding contractions evoked in eight rats. Switching on the stimulation when the bladder pressure increased 10 cmH_2O above the baseline had no effect on the contraction and the pressure continued rising progressively culminating in a voiding (Figure 1). Comparison of urodynamic parameters in control and stimulation CMGs showed no significant differences in p_{max} or p_{basal} (Table 1). BC at which a voiding contraction started was comparable. The rise time of pressure from baseline to the point of beginning of a voiding contraction and the exponential decay of pressure after a voiding also remained unaffected by stimulation (Table 1).

3.2 | Conditional dorsal penile nerve stimulation

Three out of 10 rats were used for optimization of the algorithm. The stimulation was switched on manually and the detection software was updated dynamically based on the results. Urodynamic parameters were not calculated.

In the other seven rats, stimulation was switched on automatically based on the AUC threshold determined from the control CMGs in these rats. In a total of 37 CMGs, 106 contraction inhibitions were attempted, out of which 74 (70%) were inhibited "on-time" (Figure 3). The bladder pressure at which the automatic stimulation started was 8 ± 5 cmH_20. In the other 30 measurements, the automatic stimulation was "late" (Figure 4) and the AUC threshold triggered the stimulation when the pressure had reached 20 cmH_2O or more. Only in 13 of these measurements leakage occurred, while in the other 17 voiding was still inhibited. BC in conditional stimulation CMGs increased 45% compared to that of controls (Table 2).

The p_{max} at the beginning of a voiding contraction did not differ between the control and stimulation measurements (Table 2). However p_{max} in control CMGs was found to be significantly higher when compared to that of the first inhibited contractions (Table 3, Figure 2). As a result of the continuous increase in volume, the p_{max} at the last inhibited contraction was higher than that at the first inhibited contraction (Figure 2). The ratio $p_{basal\ (after\ stimulation/before\ stimulation)}$ was higher in the first inhibited contractions than in the last inhibited ones (Table 3). In 80% of the first inhibited contractions the bladder pressure returned to 80% of the $p_{basal\ before\ stimulation}$ value at the start of filling, whereas only 60% of the last inhibited contractions returned to 80%.

4 | DISCUSSION

4.1 | Conditional tibial nerve stimulation

In anaesthetized animal models, tibial nerve stimulation has been reported to inhibit bladder contractions, resulting in an

TABLE 1 Mean ± SD of the estimated parameters in filling evoked contractions in control ($n = 28$) and conditional tibial nerve stimulation ($n = 50$) cystometrograms

	Control	Conditional tibial stimulation	*P*-value
Rise time (s)	6.6 ± 4.3	5.5 ± 2.6	0.2
Maximum pressure (cmH_2O)	24.3 ± 7.0	23.4 ± 5.8	0.6
Baseline pressure (cmH_2O)	2.6 ± 1.6	2.5 ± 1.3	0.7
Pressure decay constant (s)	7.7 ± 4.4	8.4 ± 4.0	0.5
Bladder capacity (mL)	0.9 ± 0.3	1.0 ± 0.3	0.2

Difference was considered significant at $P < 0.05$.

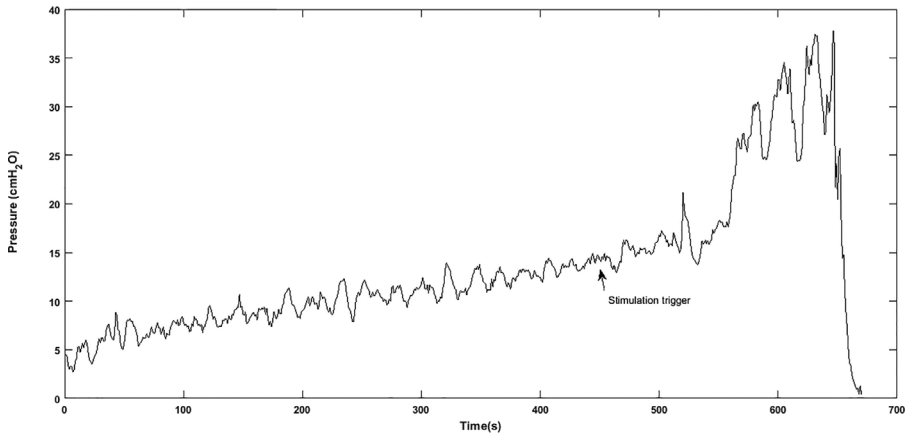

FIGURE 4 An example of a "Late" stimulation

increase in bladder capacity.[18,19] We hypothesized that, similar to acute continuous tibial stimulation; acute conditional stimulation might also recruit a lumbo-sacral spinal cord route to produce an inhibitory effect. Unfortunately, conditional TN stimulation failed to suppress bladder contractions in all of our animals. Only limited research has been done to test conditional TN stimulation.[20,21] In a study on chronic spinal cord injured cats, the authors not only reported the ineffectiveness of acute TNS, but also found that stimulation was in fact followed by increased bladder activity.[21] A study in multiple sclerosis NDO patients reported similar negative results.[20] A potential drawback of this patient study was the maximum tolerable stimulation threshold of only 1.5T. In order to maximize the number of depolarized afferents, we used higher stimulation thresholds of up to 5T and pulse widths of 200 and 400 μs. Even these stimulation parameters did not produce any beneficial effects. It is worth mentioning that we used higher stimulation thresholds in the rats to test if surpassing the maximum tolerable stimulation threshold resulted in a positive effect of TNS. In a clinical application such high amplitudes are not feasible. To test if stimulation would affect bladder pressure dynamics during a voiding contraction, we examined the pressure rise time from the baseline to its culmination into a voiding and the subsequent exponential decay to baseline (Figure 1). There was no appreciable change in these parameters between the stimulation and control CMGs. Evidently, continuous stimulation for longer periods remains the only viable option to achieve bladder inhibition via the TN.

4.2 | Conditional dorsal penile nerve stimulation

In a recent study,[15] we reported that voiding in rats is preceded by recurrent changes in the frequency of bladder non-voiding activity. These changes were detected using a moving average Fast-Fourier Transform algorithm. The algorithm was tested offline on pre-recorded pressure data and we were able to demonstrate a high success rate of 90% in detecting frequency changes occurring from 100 s before a voiding contraction. In our current study we aimed at expanding the developed algorithm to real-time, and to show that the frequency of NVCs can be used as a trigger for closed-loop conditional inhibition of bladder contractions via the DPN.

Conditional stimulation successfully suppressed 70% of the contractions evoked by bladder filling. The resulting 45% increase in BC is well supported by other conditional stimulation studies.[5] A common observation in conditional stimulation studies is that bladder contractions can only be inhibited up to a certain bladder volume.[5] In our study too, as the bladder volume increased with filling, an increase in p_{max} was seen from the first inhibited contractions to the last inhibited contraction, which was followed by voiding (Figure 2). An impact of increasing bladder volume was also seen in the efficiency of stimulation in bringing down the pressure to baseline. In 80% of the first inhibited contractions p_{basal} returned to 80% of the pre-stimulation level, whereas, only 60% of the last inhibited contractions returned to 80% level. Presumably when the volume threshold for voiding is

TABLE 2 Mean ± SD of the estimated parameters in control and conditional DPN stimulation cystometrograms

	Control	Conditional stimulation	*P*-value
p_{max} (cmH_2O)	35.0 ± 11.3	37.4 ± 16.6	0.2
Bladder capacity (mL)	1.1 ± 0.7	1.6 ± 0.3	0.01*

TABLE 3 Mean ± SD of the estimated parameters in control and conditional DPN stimulation CMGs

	Control	First inhibited contraction	Last inhibited contraction	*P*-value
p_{max} (cmH_2O)	35.0 ± 11.3	31.1 ± 17.0	-	0.04*
p_{max} (cmH_2O)	-	31.1 ± 17.0	36.5 ± 15.2	0.2
Ratio, $p_{basal\ (after\ stimulation/before\ stimulation)}$	-	1.06 ± 1.04	0.53 ± 0.4	0.01*

As the filling progressed, bladder contractions occurred and were subsequently inhibited until voiding occurred. For each cystometrogram in which stimulation was done, the following parameters were calculated for the first inhibited contraction and the last inhibited contraction. Statistical difference shows the difference between shown values.

reached, the bladder afferent activity overcomes the inhibitory influence of stimulation. One possible way to further inhibit afferent activity (and increase BC) would be to increase the stimulation amplitude to maximize afferent fiber recruitment, however, due to the limitation of the maximum tolerable threshold in patients, the window for stimulation parameters remains narrow.[22]

To realize an implantable neural prosthesis, various biosignals originating from the lower urinary tract have been proposed to detect the early onset of bladder contractions. Research has been done to identify the electrical activity of the bladder,[8] the anal,[10] and the urethral sphincter muscles[9] as a trigger event for conditional stimulation. These methods are hindered by the difficulties in recognizing whether the recorded bladder EMG is a true signal or just an electromechanical artifact and by the high rate of false positive detections in anal and urethral EMG.[9,23] Electric activity of pudendal, pelvic, and sacral nerves has also been proposed for conditional stimulation.[11–13] These methods, however, suffer from low signal amplitude and poor signal-to-noise ratio. A viable solution, that has been studied extensively, would be to use a certain bladder pressure (eg, 10-15 cmH_2O) as a trigger level, which is resilient to the above mentioned complications due to its relatively strong signal strength. However, switching on stimulation when the pressure has already reached a high level might cause failure in inhibition as the rapid rise in pressure would probably result in an extensive increase in afferent firing, resulting in voiding.[24] The key feature of our AUC threshold method is that stimulation started at bladder pressure levels as low as 3 cmH_2O, long before the commonly used threshold of 10-15 cmH_2O is reached. In a conditional stimulation study,[20] urgency was reported by patients ~5 s before the pressure rose to 10 cmH_2O. Although the sensation of urgency cannot be simulated in animal models, our early-alert system has the potential allow a controlled bladder emptying in patients with detrusor overactivity. Our approach of starting the stimulation at any AUC value above the stimulation threshold is rather unadaptive to random changes in frequency due to movement artifacts. This was manifested in the 70% success rate of the algorithm as 30% of the stimulations were triggered at a false stimulation threshold. An adaptive algorithm which takes into account irrelevant changes in frequency in combination with the conventional amplitude trigger technique would further improve the performance and robustness of the method.

The use of an anesthetized animal model restricts the drawing of parallels to humans. Nonetheless, non-voiding activity has now been accepted as an intrinsic bladder property rather than an anomaly and has been reported in (un) anesthetized animals as well as in humans.[25] To utilize this property as a trigger event for conditional stimulation in detrusor overactivity patients, further research is warranted on the pathological changes in non-voiding activity. Our method relies on continuous recording of the bladder pressure. In our experiments this was done via a catheter, which is not an acceptable option in clinical settings. Novel techniques are necessary to eliminate this need of indwelling catheterization and to realize a clinical application of our method.

The current study investigated the effect of conditional stimulation in a normal (non-overactive) rat model. Due to differences in non-voiding activity, different results might occur if the same technique is applied to an animal model of neurogenic bladder. In a recently initiated study,[26] a significant power difference in a specific frequency range was found in intravesical pressure recordings from normal and detrusor overactivity in four patients. The results of our present study combined with this pilot study could contribute to the realization of an implantable automatic neurostimulator in a closed-loop system.

5 | CONCLUSIONS

Conditional TN stimulation in anaesthetized rats did not inhibit bladder contractions evoked by filling. Automatic stimulation of the DPN triggered by an increased frequency of bladder non-voiding activity resulted in successful bladder inhibition at a low bladder pressure level and a consequential increase in BC. This inhibition at low pressure might prevent patients from experiencing urgency sensations. The presented technique could provide a robust detection system, contributing to the realization of an automatic closed-loop electrical stimulator.

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How to cite this article: Choudhary M, van Mastrigt R, van Asselt E. The frequency spectrum of bladder non-voiding activity as a trigger-event for conditional stimulation: Closed-loop inhibition of bladder contractions in rats. *Neurourology and Urodynamics*. 2018;37:1567–1573. https://doi.org/10.1002/nau.23504