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ORIGINAL PAPER

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Afferent bladder nerve activity in the rat: a mechanism for starting and stopping voiding contractions

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Abstract The objective of this work was to study the relation between afferent bladder nerve activity and bladder mechanics and the mechanisms that initiate and terminate bladder contractions. Bladder nerve activity, pressure and volume were recorded during the micturition cycle in the rat. The highest correlation was found between afferent nerve activity and stress (pressure×volume). Afferent nerve activity depended linearly on stress within 6%, and both slope and offset were independent of the bladder-filling rate. The levels of afferent bladder nerve activity at the onset and cessation of efferent firing to the bladder were highly reproducible with coefficients of variation of $\leq 17\%$. We propose a model in which afferent activity is proportional to bladder wall stress, and bladder contraction is initiated when afferent activity exceeds a threshold due to an increasing pressure and volume. The contraction continues until afferent activity drops below a threshold again as a result of a decreasing volume.

Keywords Bladder nerve activity · Voiding · Trigger mechanism

Introduction

Two phases can be distinguished in the micturition cycle. In the storage phase, urine is collected in the bladder, bladder pressure is low and urethral pressure is high enough to prevent leakage. In the voiding phase, urine is expelled from the bladder, and this involves bladder contraction and urethral relaxation [9]. Both processes are regulated by the central nervous system. Several models of the innervation of the lower urinary tract in which physiological and neuronal properties are combined have been proposed [10, 12, 40]. Detailed knowledge of the nervous control of the lower urinary tract may enable the development of new diagnostic methods or treatments for neurogenic dysfunction.

In general, bladder afferent fibers conveying information from the bladder to the spinal cord can be divided into C fibres (conduction velocity < 1.3 m/s) and Aδ fibres (conduction velocity ≥ 1.3 m/s). The activity in these fibres has been related to bladder pressure [2, 19, 27, 34], wall tension [8, 10, 30] and stress [32]. Occasionally, receptor area related activity [8] or volume receptors [28, 29] have been reported. Modelling based on the recording of compound nerves is useful because the relation between afferent nerve activity and the physical state of the bladder improves once the activity of more units is included [44].

We have previously shown that afferent bladder nerve activity triggers efferent firing to the bladder. We assumed that afferent activity was proportional to bladder pressure [39]. With increasing bladder volume, the pressure increases and thus the afferent activity increases until it exceeds a threshold. Then a contraction is started, the pressure rises further and the afferent activity increases further. This positive feedback mechanism may facilitate the micturition reflex. However, this model, like many others, fails to explain the end of the contraction: the cessation of efferent bladder nerve activity. A better understanding of this neural mechanism is relevant in patients with hypertrophy of the prostate (BPH). In these patients the bladder contraction often fades before the bladder is empty.

The present study was done to define the quantitative relations between afferent activity and bladder pressure, bladder volume and the derived variables: strain, bladder surface, bladder wall tension, stress and estimated stress. The variable that correlated best with afferent nerve activity was used to investigate the levels of afferent nerve activity at the onset and cessation of efferent firing.

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Methods and materials

Surgery

Male Wistar rats (16 animals with a mean weight of 451 ± 47 g) were anaesthetised with urethane (1.2 g/kg) intraperitoneally [22] and placed on a heated undercover. An abdominal midline incision was made to access the bladder. The abdominal wall was tied to a frame to create a basin, which was filled with warm paraffin oil during the measurements. A fan like pattern of very thin fragile nerves could be seen between the major pelvic ganglion and the bladder. One of these bladder nerves [25] on the left hand side was dissected at two sites a few mm apart (Fig. 1). There was no branching between the two dissection sites. The bladder nerve was guided over a bipolar platinum-iridium electrode at the peripheral site and in some cases transected at the central site to exclude all efferent nerve activity. In five animals, nerve activity could not be distinguished from background noise (the estimated signal to noise ratio $SNR_{est} < 1$ in intact nerves or < 0.5 in transected nerves) [19]. In three animals, the bladder did not contract after filling. In four animals, nerve activity was successfully recorded in intact nerves. In two of these and in four other animals, bladder nerves were successfully transected and recorded. The recorded nerve signal was amplified by a DISA 15C01 EMG amplifier (amplification range: 50,000–200,000) and band-pass filtered (Bessel, fourth order; Krohn-Hite model 3944). The pass band was set from 100 Hz to 2 kHz, allowing measurement of spikes with pulse widths down to 0.5 msec [33]. A 24 Ga angiocatheter was inserted near the top of the bladder. The bladder was filled with room-temperature saline while the intravesical pressure was measured using a disposable pressure transducer and a Statham SP1400 blood pressure monitor. The bladder was filled with a Harvard Apparatus (Millis-Massachusetts) infusion pump at infusion rates of 0.2, 0.5, 1 or 2 ml/min. Measurement series including several micturition cycles lasted 79 ± 46 min. The pressure and bladder nerve signals (Fig. 2) were read into a personal computer at sample rates of 10 Hz and 25 kHz, respectively. After the experiments the bladder was removed to determine the tissue volume (V_t) and weight (m).

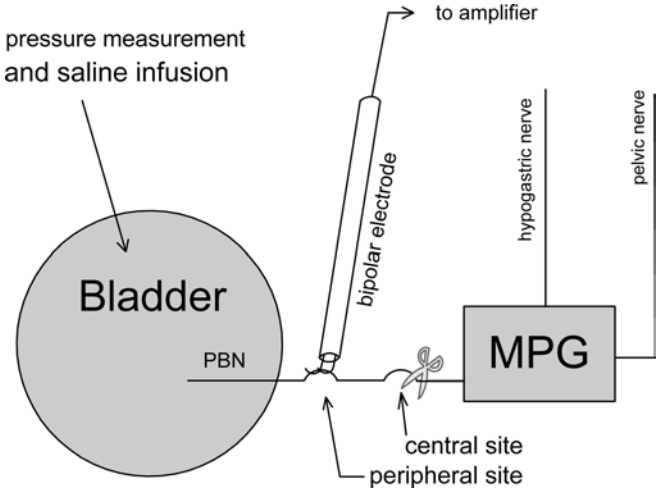

Fig. 1 A schematic drawing of the measurement electrode configuration. The bladder was filled with saline through a needle inserted in the bladder until voiding contractions occurred. Bladder pressure was also measured through this needle. Postganglionic bladder nerves (*PBN*s) were dissected from the underlying tissue between the bladder and the major pelvic ganglion (*MPG*) at two sites. The peripheral site was used for recording nerve activity with a bipolar platinum iridium electrode. For afferent nerve recordings, the bladder nerve was transected at the central site

Modelling

The mean value of the rectified nerve signal in 100 ms intervals was calculated as a measure for total nerve activity (NA) [1, 13, 19, 43]. The period $t_1 < t < t_2$ during

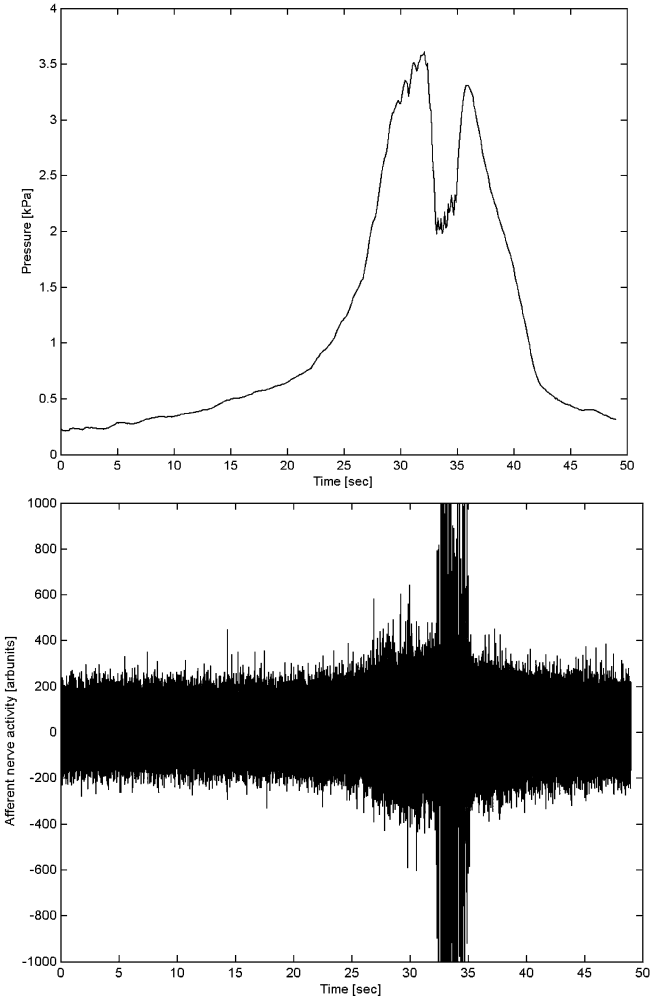

Fig. 2 An example of a recording of bladder pressure and nerve activity during a voiding contraction. The bladder nerve was transected at the central site in order to record afferent nerve activity only

which there was flow through the urethra was excluded from analysis because nerve activity could not adequately be determined (see Fig. 3). During this period rhythmic urethral sphincter contractions caused oscillations in the bladder pressure and movement of the electrodes recording nerve activity [19].

The afferent nerve activity (NA_{aff}) was related to the bladder pressure (P), the bladder volume (V) and the derived variables: strain (ϵ), bladder surface (A_{det}), bladder wall tension (T), stress (σ) and estimated stress (σ_{est}). For the calculation of these variables, the bladder was approximated to a sphere with volume V and radius r [41],

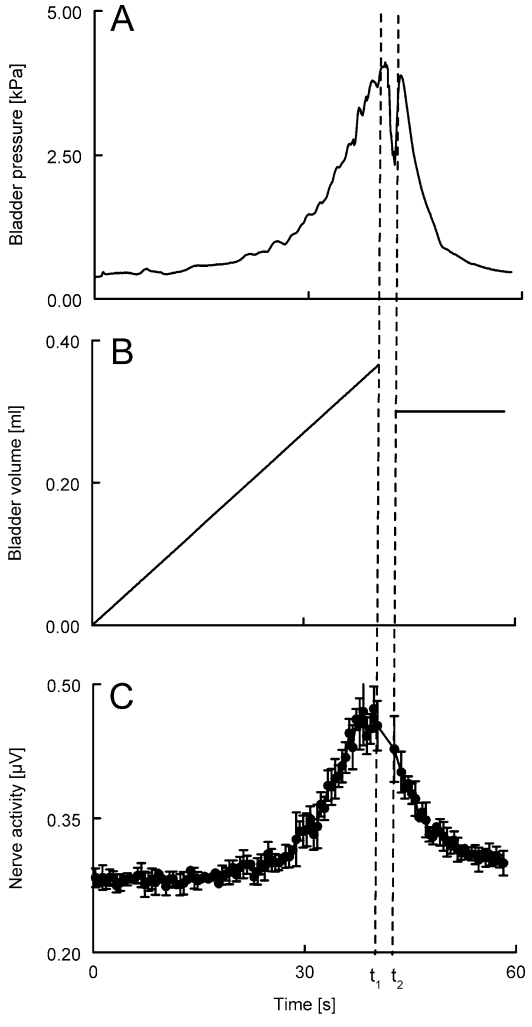

Fig. 3 Examples of the measured **A** bladder pressure, **B** volume, and **C** afferent bladder nerve activity. The bladder was manually emptied before the measurement. At $t = 0$ the infusion pump was started and the bladder was filled until a voiding contraction occurred. Then the pump was switched off. After the measurement the residue was determined. The period with flow through the urethra ($t_1 < t < t_2$) was excluded from the analysis because there were movement artifacts in the measured nerve signal in this episode and the bladder volume was not exactly known. Nerve activity was calculated ten times per second. To reduce the influence of noise, the means of five consecutive values were displayed. *Error bars* depict standard deviations

$$\varepsilon = (l - l_0)/l_0 = \sqrt[3]{V/V_0} - 1 \quad (1)$$

$$A_{det} = 4\pi r^2 = 4\pi \cdot (3 \cdot V/4\pi)^{2/3} \quad (2)$$

T: proportional to $P \cdot r$ (see appendix) (3)

$$\sigma = 3 \cdot P \cdot V/2 \cdot V_t + P/4 - P_{out} \text{ (see appendix)} \quad (4)$$

σ_{est}: proportional to $P \cdot V$ (see appendix) (5)

Here l is the length of a certain bladder segment with initial length l_0, V_0 is the initial bladder volume, V_t is the bladder tissue volume and P_{out} is the (constant) atmospheric pressure.

$$\text{relative error} = \frac{\Sigma|NA_{aff} - (\text{slope} \cdot \textit{variable} + \text{offset})|}{\Sigma NA_{aff}} \times 100\% \quad (6)$$

We calculated the correlation coefficients of NA_{aff} and the seven variables, fitted linear relations ($NA_{aff} = \text{slope} \cdot \textit{variable} + \text{offset}$) and calculated the relative fit error (Eq. 6).

The intact nerve measurements were analysed with a previously described model [19], which enabled estimation of afferent and efferent contributions to the nerve signal. This model was slightly modified:

1. It was assumed that all nerve activity after t_2 was afferent, which enabled determination of the relation between NA_{aff} and the selected variable (not necessarily the bladder pressure as assumed in the original model).
2. It was assumed that NA_{aff} depended linearly on this variable. Slope and offset were determined from the episode $t > t_2$. Subsequently NA_{aff} was calculated for $t < t_1$.
3. Linear addition of efferent and afferent nerve activity was assumed, which enabled estimation of NA_{eff} by subtracting NA_{aff} from the measured total nerve activity for $t < t_1$.

Measurements

The bladder was filled with saline until a voiding contraction occurred. Bladder pressure and bladder nerve signals were recorded throughout the micturition cycle. After each measurement the residual volume was determined.

In centrally transected nerves, the recorded nerve signal was purely afferent. Correlation coefficients were calculated for NA_{aff} with P, V, A_{det}, ε, T, σ, and σ_{est} (Eqs. 1–5). Linear models ($NA_{aff} = \text{slope} \cdot \textit{variable} + \text{offset}$) were fitted for the best correlating variables. In each rat, both the differences between the seven correlation coefficients and between the fit errors were statistically verified using a paired *t*-test.

To investigate the effect of the filling rate on the evoked NA_{aff}, linear relations were fitted to data obtained at four different filling rates (0.2, 0.5, 1 and 2 ml/min). In each rat, the slopes and offsets of the fitted lines were normalised to their values at 1 ml/min, and averaged. The filling rate dependence of slopes and offsets was statistically tested by one-way analysis of variance.

Furthermore, slope and offset of the relation between NA_{aff} and the best correlating variables during $t > t_2$ were determined, and the nerve activity during $t < t_1$ was calculated based on these parameters to assess the error in the calculation of afferent activity in the intact nerve measurements.

Finally, we estimated the level of afferent nerve activity at the start of bladder contraction ($t_{0.est}$) by determination from the nerve signal and by calculation from the appropriate linear relation. It was compared with the afferent activity at t_2.

In intact nerves, measurements were done to determine the level of afferent nerve activity at the beginning and end of efferent firing. Afferent and efferent contributions to the measured combined nerve signal were calculated with the model, and NA_{aff} was assumed to be proportional to P×V. The start of bladder contraction was defined as the moment at which efferent firing to the bladder was initiated. Previously a model has been fitted that assumed no efferent activity until t_0, and then a linear increase until the voiding started. Efferent nerve activity to the bladder started when the afferent activity (which was related to pressure in that study) exceeded a certain threshold [39]. In the present study, we recalculated this threshold, with afferent activity now proportional to P×V.

The exact ending of efferent firing could not be determined because of the movement artifacts recorded during flow. To estimate the level of afferent activity at the end of efferent firing, we calculated afferent nerve activity at t_2 (see Fig. 3). The reproducibility was assessed by the coefficients of variation [(SD/mean)×100%]. Both thresholds were compared using a paired *t*-test.

Experiments were carried out as outlined in the 'Erasmus University of Rotterdam Guidelines for the Care and Use of Laboratory Animals', which in general follows the NIH 'Guide for the Care and Use of Laboratory Animals'. All data are presented as mean ± SD. Differences were considered statistically significant if $P < 0.05$.

Results

The bladder was filled with saline until a voiding contraction occurred. The average filled volume was 0.82 ± 0.19 ml and the residual volume 0.58 ± 0.22 ml. Mean bladder weight (m) and tissue volume (V_t) were 0.21 ± 0.06 g and 0.23 ± 0.06 cm^3 (average density 0.95 ± 0.23 g/cm^3).

Centrally transected nerve measurements

In six rats (93 measurements), afferent bladder nerve activity was recorded during $t < t_1$ and $t > t_2$ and correlated to bladder pressure (P), volume (V), strain (ϵ), surface (A_{det}), tension (T), stress (σ) and σ_{est} (pressure×volume) (Fig. 4). In five of the six rats (86 measurements) the bladder was filled at four different filling rates: 0.2, 0.5, 1 and 2 ml/min. All measurements were pooled because the filling rate had no significant effect on the average correlation coefficients, slopes, offsets or fit errors. The average coefficients of variation were 33% for slope and 8% for offset.

The best correlation was found between nerve activity and (estimated) stress (Table 1). Nerve activity always correlated significantly better with stress than with strain, surface and volume (paired *t*-test: $P < 0.05$). In 83% of the rats, nerve activity correlated significantly better with stress than with pressure, and in 67% also better than with tension.

Linear relations were fitted between afferent nerve activity and the four best correlating variables ($NA = slope \cdot variable + offset$) (Table 1). Stress (and P×V) showed the smallest fit error, significantly smaller than for pressure in 83% of the animals, and in 33% also significantly smaller than for tension.

In each measurement, fit errors using stress or P×V were equal, offsets differed by less than 0.25% and slopes differed by a fixed factor equal to $3/2V_t$ within 5%.

We also calculated the slopes and offsets for pressure, tension and stress at $t > t_2$ and estimated NA_{aff} during $t < t_1$. The thus estimated NA_{aff} was compared to the measured NA_{aff}. On average the errors were 4.9% for pressure, 4.2% for tension and 3.4% for stress (Fig. 5).

Finally, we investigated the levels of NA_{aff} from the bladder at the onset and cessation of efferent bladder nerve firing. Onset ($t_{0.est}$, the moment when the bladder pressure started to increase progressively) and cessation (t_2, the moment when the bladder pressure started to decrease) were estimated (Fig. 6). $NA_{aff}(t_{0.est})$ was 0.80 ± 0.67 μV and $NA_{aff}(t_2)$ was 0.86 ± 0.59 μV. Both $NA_{aff}(t_{0.est})$ and $NA_{aff}(t_2)$ showed excellent reproducibility (coefficients of variation $9 \pm 3\%$ and $15 \pm 6\%$). In four rats NA_{aff} at $t_{0.est}$ and t_2 did not differ significantly while in two rats NA_{aff} was significantly higher at t_2 than at $t_{0.est}$ ($P < 0.001$).

Intact nerve measurements

In four rats (23 measurements) intact nerves were recorded (Fig. 7). Since there was no efferent activity during the pressure decline after t_2 [19], we calculated the relation (slope and offset) between NA_{aff} and P×V during this period. Then we calculated afferent nerve activity during $t < t_1$. Subtraction from the total nerve activity yielded an estimate of the efferent contribution.

The level of afferent activity at the onset of efferent firing $NA_{aff}(t_0)$ was 1.71 ± 0.6 μV and its coefficient of

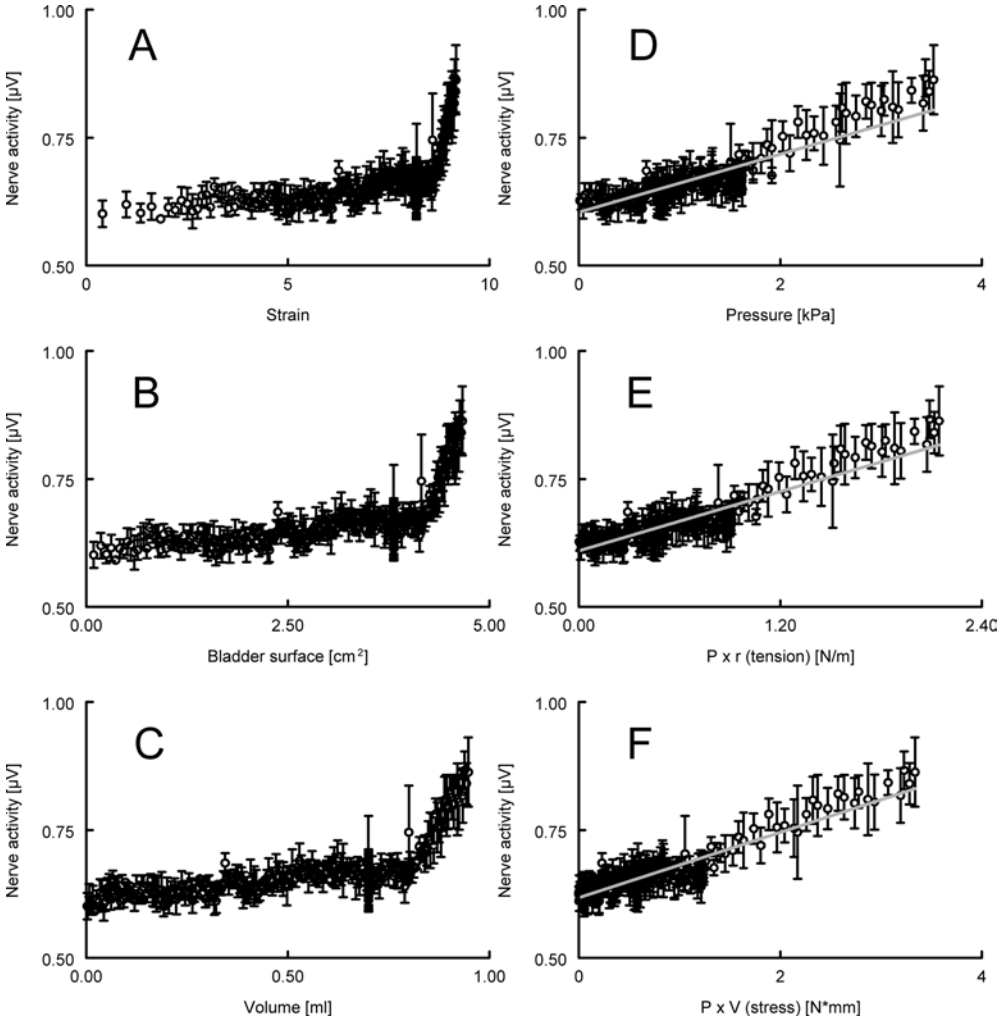

Fig. 4 An example of afferent bladder nerve activity measured during one filling/voiding cycle, related to: **A** strain, **B** bladder surface, **C** volume, **D** pressure, **E** P×r (tension) and **F** P×V (stress), shown in order of increasing correlation coefficient (on average $r^2 = 0.55$, 0.60, 0.64, 0.78, 0.80, and 0.81, respectively). Linear equations were fitted to the relations between nerve activity and the three best correlating mechanical variables: pressure, tension and stress

Table 1 Correlation coefficients and linear fit parameters and errors. Afferent bladder nerve activity was related to several mechanical variables. Shown are correlation coefficients and linear fit parameters and errors of the variables. The only difference between stress and P×V was the value of the slopes that differed by a factor $3/2V_t$ in each rat (V_t is the individual bladder tissue volume). Also shown is the percentage of animals in which a smaller correlation coefficient or a larger fit error (compared with stress) was significant. Standard deviations refer to differences between rats

Variable	Correlation coefficient	Significance $< r^2$ (stress)	Slope·(10^{-2})	Offset	Relative error (%)	Significance > error(stress)
Stress	0.81 ± 0.09	-	0.38 ± 0.36	0.67 ± 0.56	6.2 ± 1.7	-
P×V	0.81 ± 0.09	-	2.2 ± 2.2	0.67 ± 0.56	6.2 ± 1.7	-
P×r	0.80 ± 0.10	67%	2.5 ± 2.1	0.66 ± 0.55	6.5 ± 1.8	33%
Pressure	0.78 ± 0.10	83%	1.3 ± 0.9	0.65 ± 0.55	6.8 ± 1.8	83%
Volume	0.64 ± 0.08	100%			8.9 ± 2.3	100%
Surface	0.60 ± 0.07	100%			9.4 ± 2.4	100%
Strain	0.55 ± 0.07	100%			9.9 ± 2.6	100%

variation $17 \pm 16\%$. The exact ending of efferent firing could not be determined due to movement artifacts during $t_1 < t < t_2$. However, after t_2, efferent activity was absent. Afferent activity at t_2, $NA_{aff}(t_2)$ was 1.89 ± 0.7 μV and also showed high reproducibility (coefficient of variation $15 \pm 12\%$).

The level of afferent activity at the end of efferent firing was significantly higher than that at the onset of efferent nerve activity (paired *t*-test: $P < 0.001$) in all animals.

Discussion

Bladder pressure, volume and nerve activity were measured during the micturition cycle in the rat. The bladder volume averaged 0.82 ± 0.19 ml when voiding started, which is similar to the ~0.6 ml [25, 39] to 0.8 ml [37] found in other studies. The residue of 0.58 ± 0.22 ml agrees with the 0.5 ± 0.5 ml found by Mallory et al. [25].

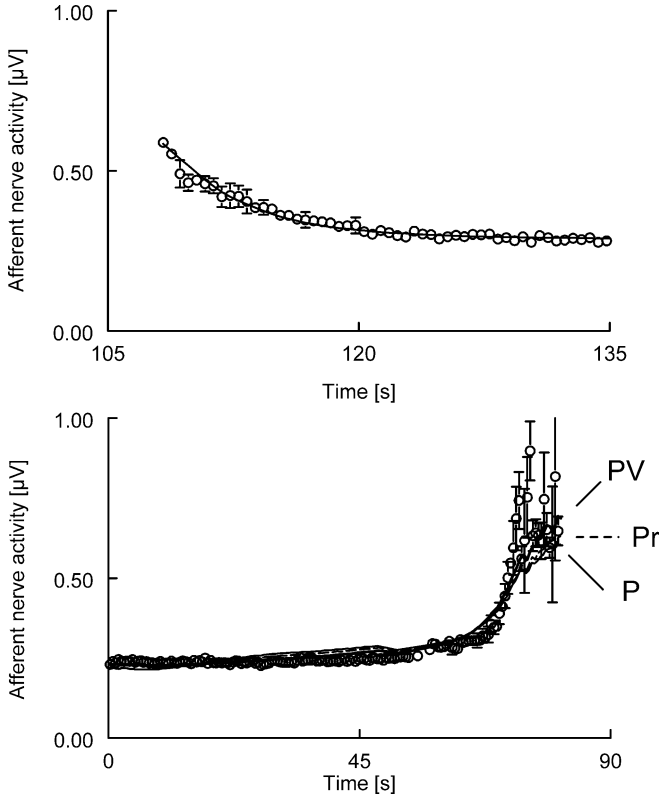

Fig. 5 *Upper panel*: taking pressure (*P*), tension (*Pr*) or stress (*PV*) as the dominant stimulus for afferent bladder nerve activity, linear equations were fitted to data collected during the pressure decline immediately after voiding ($t > t_2$ in Fig. 3). Because the volume did not change in this period, the fits are equal but the slopes differ. *Lower panel*: using the obtained parameters, nerve activity was calculated from the bladder pressure (*thin solid line*), tension (*dashed line*) and stress (*thick solid line*) during the filling phase ($t < t_1$). Calculated afferent nerve activity was compared to measured activity and differed less than 5% using either stimulus

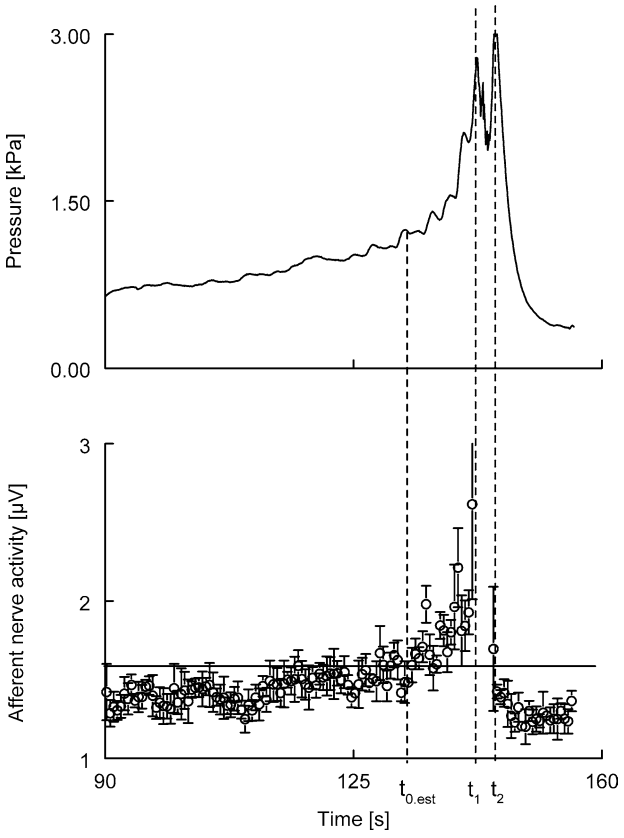

Fig. 6 In the centrally transected nerve measurements the onset of the active bladder contraction was estimated as the moment when the pressure started to increase progressively ($t_{0.est}$). The contraction was assumed to end at the pressure maximum immediately after voiding (t_2). The afferent nerve activity at $t_{0.est}$ was compared to that at t_2

The high residual volumes may be related to the relatively heavy weight of the animals used in both of these studies. The renal excretion ~0.01 [11] to 0.02 ml/min [18] was negligible compared to the imposed filling rate.

Anaesthesia influences voiding and possibly nerve activity [24, 26]. To avoid nerve activity changes due to changes in the depth of anaesthesia or due to a deterioration of the nerves, measurements were restricted to episodes of 5 min and measurement series lasted less than 120 min in all animals. This led to a minimal filling rate of 0.2 ml/min, which is higher than the physiological rate. However, a tenfold increase of the filling rate did not alter the relations that we found between afferent activity and pressure, tension and stress, suggesting that our results may be extrapolated to physiological filling rates.

The filled bladder volumes, pressures and the duration of the voiding contractions were normal. We therefore assume that the contractions were physiological and that the associated afferent and efferent nerve activity was physiological as well. However, the voided volumes were extremely small. The fact that relaxation did start presumably means that the residual volume is small enough for afferent activity to decrease.

Evoked bladder voidings in unanaesthetized rats produce complete emptying [45]. The incomplete voiding in anaesthetized rats is thought to be due to a reduced influence of higher centres on the pontine-vesico-vesical reflex [21]. Urethane has no effect on ganglionic transmission. Yaksh et al. [45] found that the volume at which the pressure starts to rise in anaesthetized animals is higher than the expelled volume in awake animals. Unfortunately, they did not test urethane, but evoking efferent activity might in general require a higher level of afferent activity in anaesthetized animals. Higher thresholds may explain larger residual volumes.

Another phenomenon important for complete voiding involves the so called intravesical pressure high frequency oscillations (IPHFOs) [6, 7]. They are presumably caused by contraction of the external urethral sphincter (EUS) since blockade of this muscle or transection of the innervating pudendal nerve leads to the absence of IPHFOs and to an increase in the residual volume [23].

It is generally known that for a normal micturition to occur urethral and bladder activity have to be highly coordinated. A variety of interneuron populations on

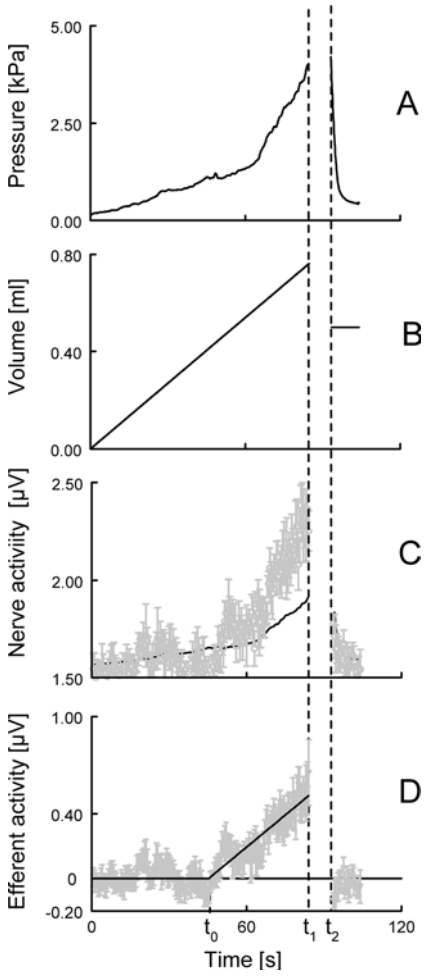

Fig. 7 An example of: **A** bladder pressure, **B** volume, and **C** and **D** nerve activity in an intact postganglionic bladder nerve during the micturition cycle. For $t > t_2$, the relation between afferent nerve activity and pressure×volume was determined (slope and offset). Then, afferent activity (*line*) was calculated for the entire measurement using the obtained equation, and subtracted from the measured total activity (*o*) (**C**). Thus efferent activity (NA_{eff}) to the bladder was calculated (∇). A model was fitted to NA_{eff}, assuming NA_{eff} is zero until t_0, and then increases linearly with time (line) (**D**). The onset of efferent firing to the bladder was defined as t_0, and the levels of afferent bladder nerve activity at t_0 and t_2 were compared. In one rat $NA_{aff}(t_0)$ equalled $NA_{aff}(t_2)$, in three rats $NA_{aff}(t_2)$ was significantly higher than $NA_{aff}(t_0)$

the sacral level participate in the coordination of parasympathetic bladder efferents with that of EUS motorneurons [36]. Efferent activity to the urethra is probably also triggered by bladder afferents [3, 16, 27]. The bursts of activity of the external urethral sphincter are probably a reflex due to bladder afferent input to the CNS. It is also suggested that during bladder filling afferent feedback from the EUS is excitatory to the bladder [17]. Under saline conditions, an intra-urethral pressure of 50 cm H_2O leads to inhibition of the bladder [38]. Buss suggested that the excitability of urethral afferents changes during micturition [5]. Whether or not flow is a prerequisite for bladder contraction to last is still under debate [17, 35].

Measurements of compound nerves lack the distinction between C- and Aδ-fibres. Using a conduction velocity of 1.3 m/s as the discriminating factor, 70% of the fibres in rat postganglionic bladder nerves are Aδ- and 30% are C-fibres [31]. Sengupta and Gebhart [34] describe low and high threshold mechanoreceptors and silent receptors, which are either C- or Aδ-fibres. However, action potentials in Aδ-fibres have been shown to have much larger amplitudes than those in C-fibres [20]. Capsaicin administration increases the bladder capacity in rats, which is generally interpreted as evidence for a role of C-fibres in the micturition reflex; however, C-fibres are not the sole pathway [24]. Moreover, some Aδ-fibres are capsaicin sensitive [14, 15, 22, 46]. Maggi and Meli [22] state that a capsaicin-resistant mechanism produces effective bladder voiding. Morrison [29] concluded that the volume receptors are mainly C-fibres that discharge during a normal distension but with higher thresholds than Aδ-fibres. Our finding that afferent bladder nerve activity is proportional to both bladder volume and pressure may indicate that we recorded both C- and Aδ-activity.

Several variables were calculated from pressure and volume: strain, bladder surface, wall tension, and stress. Stress showed the highest correlation with afferent bladder nerve activity and correlated significantly better than pressure in 83% of the animals, in 67% it was also significantly better than tension. We expected to find only very small differences because pressure and volume are closely related. In fact, if only the period before voiding was analysed, the correlation coefficients did not differ significantly. However, immediately after voiding, the pressure changed independently of the (constant) bladder volume. Analysing this episode, therefore, is necessary to enable discrimination between the influence of bladder volume and pressure on afferent nerve activity.

Figure 4 shows that the small correlation coefficients between NA_{aff} and strain, bladder surface or volume are at least partially explained by non-linearities. The relations between NA_{aff} and P, P×r or P×V are approximately linear. In agreement with an earlier study [19], we found a fit error of ~7% when afferent nerve activity was linearly related to pressure. The relation between stress and nerve activity was described by a linear function with a smaller fit error ($6.2 \pm 1.7\%$). A disadvantage of modelling stress as stimulus for afferent activity is that the calculation of stress involves the tissue volume of the bladder, which is difficult to determine accurately (coefficient of variation of tissue density is 24%). We therefore approximated stress by pressure×volume which did not influence the correlation coefficient or fit error. This approximation can be made only if $3\ V/2V_t >> 0.25$ (see appendix). During the pressure decrease after voiding, the bladder volume remained constant; thus afferent activity was proportional to bladder pressure, with a slope $a \cdot (3\ V/2V_t + 0.25)$. With a small residual bladder volume, the term 0.25 becomes dominant. Thus, with a very small residue, the

slope of the relation between P×V and NA_{aff} may be different for the periods before and after voiding. The approximation that stress is proportional to P×V may therefore be valid only if there is a substantial residual volume after voiding.

Satchell and Vaughan [32] showed that during bladder contraction afferent activity is linearly related to bladder pressure, but the slope depended on the filling rate. Due to the visco-elasticity of the bladder wall, at higher filling rates, the pressure at a given volume will usually be higher [42], which changes the pressure-volume relation and would explain the different slopes. Therefore, Satchell and Vaughan [32] suggested that afferents should be related to stress rather than pressure. Moss et al. [30] have shown that the pressure threshold for afferent firing depends on the filling rate, whereas the volume threshold did not. In our experiments, the relation between pressure and nerve activity did not depend on the filling rate. This is probably explained by the inclusion of the pressure decrease immediately after voiding when the bladder pressure changed while the volume remained constant.

In an earlier study, we also found higher afferent activity during pressure development at the onset of the voiding contraction than at comparable pressures during the pressure decrease at the end, when the bladder volume is lower [19]. To relate afferent nerve activity to bladder pressure is thus only warranted if the volume does not show major changes. Otherwise, afferent bladder nerve activity should be related to stress.

In a previous study, we showed that the level of afferent bladder nerve activity triggers efferent firing to the bladder [39]. If a certain threshold is exceeded, efferent firing starts and causes bladder contraction; subsequently the pressure rises, resulting in more afferent activity. Thus, a minor excess of the threshold results in a major increase in afferent activity. This model however, fails to explain how efferent firing ends. This problem is solved if afferent nerve activity is related to stress rather than to pressure. Again, a minor excess of the threshold results in a major increase in afferent activity, which remains far above threshold until the actual voiding is started. Then, due to a decreasing volume, afferent activity decreases until it drops below a second threshold value. Efferent activity ceases and the contraction terminates. Pressure decreases and afferent activity ends up far below threshold. Thus, in both directions, a minor excess of the threshold leads to a snowball effect and subsequent decisive threshold crossing (Fig. 8).

In the intact nerve measurements, t_0, the moment at which bladder pressure starts to increase progressively, was determined. In these experiments, we estimated the efferent contribution to the total activity by subtraction of afferent activity, which was calculated from P×V. Biró and Partridge [4] demonstrated that the nerve or muscle action potentials of independent units are linearly superimposed in compound records. The slope and offset of the relation between NA_{aff} and P×V were calculated from the episode $t > t_2$, when the measured nerve signal was entirely afferent. This introduced an error of ~5% in the estimated NA_{aff} during $t < t_1$ (6% in [19]). Fitting this model to the efferent activity resulted in a more reliable value for t_0 than in the centrally transected nerve measurements.

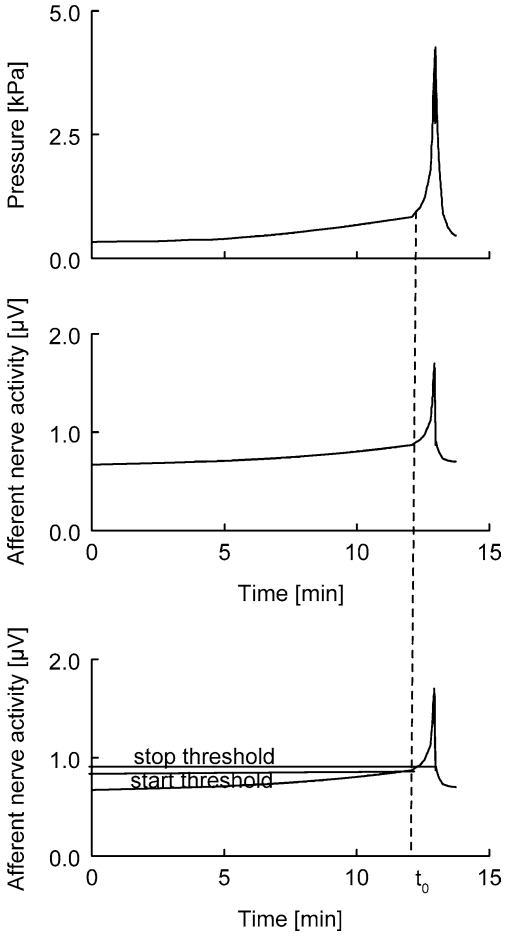

Fig. 8 Simulation of pressure and volume in a slowly filling bladder. At t_0, the active bladder contraction starts and the pressure rises. During the actual voiding the volume decreases and rapid sphincter contractions cause an oscillatory pattern in the bladder pressure. These parts of the graphs are *dotted* as they can only be estimated in this period. Afferent nerve activity was calculated as $0.022 \cdot P \times V + 0.67$. The levels of afferent activity at the onset and cessation of efferent firing to the bladder are indicated as *start threshold* and *stop threshold*. Crossing the *start threshold* causes a large increase in afferent activity and crossing the *stop threshold* causes the afferent activity to further decrease, which makes the system bi-stable

Examination of the level of afferent activity at the cessation of efferent firing revealed that it was significantly higher than the level at the onset of efferent firing in all of the intact nerves and in two of the six transected nerves. A higher threshold at cessation might be explained by adaptation to a higher afferent level of the neurons that induce efferent firing; more afferent activity may be needed to continue the efferent firing.

In this study we describe the relation between afferent bladder nerve activity, efferent bladder nerve activity

and bladder wall stress. We propose a model in which afferent activity is proportional to stress. Bladder contraction is initiated by exceeding an afferent threshold due to increasing pressure and volume and then continues until afferent activity drops below a threshold as a result of the decreasing volume.

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Appendix

Calculation of bladder wall tension (T) and stress (σ).

The bladder is approximated to a sphere with radius R (Fig. 9):

Tension

The ring area, A_{ring}, can be calculated as

$$A_{ring} = 2\pi \cdot R \cdot \cos\alpha \cdot R \cdot d\alpha = 2\pi R^2 \cdot \cos\alpha \cdot d\alpha \tag{A1}$$

The force on this ring, F_{ring} equals $P \cdot A_{ring}$. P is bladder pressure.

The vertical component, $F_{ring,up}$, equals $F_{ring} \cdot \sin\alpha$. Thus:

$$F_{ring,up} = 2\pi \cdot R^2 \cdot P \cdot \cos\alpha \cdot \sin\alpha \cdot d\alpha \tag{A2}$$

Integration yields the force that drives the two semispheres apart, F_{up}:

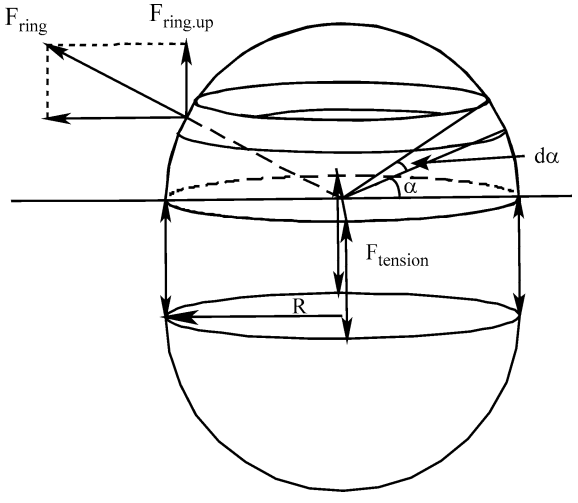

Fig. 9 Calculation of bladder wall tension and stress. The bladder is approximated to a sphere with radius *R*. The force that drives the two semi-spheres apart is balanced by the force keeping them together: $F_{tension}$. Tension is calculated as $F_{tension}$/(circumference of cross section) and stress as $F_{tension}$/(area of cross section)

$$\begin{aligned} F_{up} &= \int_0^{\pi/2} \pi \cdot R^2 P \cdot 2\sin\alpha\cos\alpha \cdot d\alpha \\ &= \pi \cdot R^2 P \sin^2\alpha|_0^{\pi/2} \\ &= \pi \cdot R^2 \cdot P \end{aligned} \tag{A3}$$

The force that keeps both semispheres together, $F_{tension}$, equals F_{up}. Wall tension is defined as $F_{tension}$ divided by the circumference of the cross section:

$$T = \frac{\pi \cdot R^2 P}{2\pi \cdot R} = P \cdot R/2 \tag{A4}$$

Stress

Stress (σ) is defined as $F_{tension}$ divided by the area of the cross section. If d is the wall thickness, this area equals $\pi(R+d)^2 - \pi R^2 = 2\pi Rd + \pi d^2$. Assuming that $d << R$ yields:

$$\sigma_{thinwall} = \frac{\pi \cdot R^2 P}{2\pi \cdot Rd} = \frac{PR}{2d} \tag{A5}$$

However, when the bladder is almost empty this approximation is not valid.

With a non-negligible wall thickness, the tangential stress in the wall at radius r can be calculated [41]:

$$\sigma(r) = \frac{P_{in}r_{in}^3 - P_{out}r_{out}^3}{r_{out}^3 - r_{in}^3} + \frac{r_{in}^3 r_{out}^3}{2r^3} \cdot \frac{P_{in} - P_{out}}{r_{out}^3 - r_{in}^3} \tag{A6}$$

$$\sigma(r) = \frac{P_{in}r_{in}^3}{2r^3} \cdot \frac{r_{out}^3 + 2r^3}{r_{out}^3 - r_{in}^3} - \frac{P_{out}r_{out}^3}{2r^3} \cdot \frac{r_{in}^3 + 2r^3}{r_{out}^3 - r_{in}^3} \tag{A7}$$

With P_{in} = pressure inside sphere; P_{out} = pressure outside sphere; r_{in} = inner radius; r_{out} = R = outer radius.

With V = bladder volume and V_t = tissue volume:

$$V_t = {}^4\!/_3\pi\left(r_{out}^3 - r_{in}^3\right) \Leftrightarrow \left(r_{out}^3 - r_{in}^3\right) = \frac{3V_t}{4\pi} \tag{A8}$$

$$V = {}^4\!/_3\pi r_{in}^3 \Leftrightarrow r_{in}^3 = \frac{3V}{4\pi} \tag{A9}$$

with (A.7), (A.8) and (A.9):

$$\sigma(r_{in}) = \frac{3P_{in}}{2V_t} \cdot \left(V + \frac{V_t}{3}\right) - \frac{3P_{out}}{2V_t}(V + V_t) \tag{A10}$$

$$\sigma(r_{out}) = \frac{3P_{in}}{2V_t} \cdot (V) - \frac{3P_{out}}{2V_t}(V + {}^2\!/_3V_t) \tag{A11}$$

$$\sigma(r_{out}) = \sigma(r_{in}) - {}^1\!/_2P_{in} + {}^1\!/_2P_{out} = \sigma(r_{in}) - {}^1\!/_2P \tag{A12}$$

Mean stress in bladder wall:

$$\sigma = [\sigma(r_{out}) + \sigma(r_{in})]/2 \tag{A13}$$

from (A.12) and (A.13):

$$\begin{aligned}\sigma &= \sigma(r_{in}) - {}^1\!/_4 P \\ &= \tfrac{3}{2V_t}(P_{in} - P_{out})V + \tfrac{1}{2}P_{in} - \tfrac{3}{2}P_{out} - \tfrac{1}{4}P \qquad \text{(A14)}\end{aligned}$$

$$= \tfrac{3}{2V_t} \cdot PV + \tfrac{1}{4}P - p_{out} \qquad \text{(A15)}$$

Where $P = P_{in} - P_{out}$, and P_{out} is considered a constant. The term ${}^1\!/_4 P$ becomes negligible if $(3PV/2V_t)/0.25P > 10$, i.e. if $6\ V/V_t > 10$.

In rats V_t averages 0.2 cm^3. Thus, the pressure term is negligible if $V > 0.33$ cm^3 and should be accounted for only at low bladder volumes.

If nerve activity is proportional to stress then:

$$\begin{aligned}\mathrm{NA} &= a \cdot \mathrm{stress} + b = a(3V/2V_t + {}^1\!/_4) \\ &\quad \cdot P - [a \cdot P_{out} + b] = a(3V/2V_t - {}^1\!/_4) \cdot P + b' \qquad \text{(A16)} \\ &\text{with } b' = -a \cdot P_{out} - b\end{aligned}$$

Or under the above assumption:

$$\begin{aligned}NA &= a(3/2V_t) \cdot PV + b' = a' \cdot PV + b \\ &\text{with } a' = a(3/2V_t)\end{aligned} \qquad \text{(A17)}$$

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