Hypothesis / aims of study
The pathogenesis of the overactive bladder (OAB) syndrome remains unclear, but the symptoms are attributed to detrusor muscle overactivity: a myogenic mechanism has been proposed as one possible mechanism (1). Furthermore, unprovoked, spontaneous muscle activity is increased in patients with overactive bladders (2,3), as well as in animal models of detrusor overactivity. However, the cellular basis for enhanced spontaneous muscle activity in detrusor from overactive bladders has never been explored. In particular whether the spontaneous activity originates from smooth muscle itself or other cell types. From a mechanistic point of view, the role of intracellular Ca"^2+" is of particular importance, as it is the main determinant of detrusor contractility. The objective of the current study was to test the hypothesis that enhanced spontaneous Ca"^2+" oscillations and electrical activity in detrusor smooth muscle is associated with detrusor overactivity by examining samples from pathologically overactive bladders and control patients.

Study design, materials and methods
Human detrusor samples were obtained from patients undergoing surgical procedures, with stable bladders and idiopathic (IDO) or neurogenic (NDO) detrusor overactivity. Detrusor smooth muscle cells were isolated by disruption in a collagenase-based medium. The viability of the cells was confirmed by trypan blue extrusion and the response to physiological stimuli. Dissociated detrusor myocytes were loaded with Ca"^2+"-sensitive fluorochromes Fura-2 AM and intracellular Ca"^2+" concentration ([Ca"^2+"]) was measured using epifluorescence microscopy. Electrophysiological recordings were made with patch-type pipettes filled with an intracellular solution. For simultaneous recording of cellular [Ca"^2+"], and electrical activity, Fura-2 K_0 was included in the pipette medium and dialysed into the cell. Cells were continuously superfused with a physiological saline (Tyrode's solution), gassed with 95% O_2 : 5%CO_2, at 37°C, pH7.4. The magnitudes of autonomous, spontaneous [Ca"^2+"], rises were quantified as the time-averaged area under the curve for the duration of observation. Data are means±s.e.m. Student's t-test examined differences between data sets and χ^2-test (Fisher's exact test) between two incidences. The null hypothesis was rejected at p<0.05.

Results
Basal Ca"^2+" concentration (resting [Ca"^2+"]) was 66±3nM in cells from stable bladders (n=196 cells) and was increased to 75±4nM in IDO (n=168 cells, p<0.05) and 76±5nM in NDO samples (n=88 cells, p<0.05). Unprovoked, spontaneous rises of [Ca"^2+"], were observed in a proportion of cells, of varying amplitude, duration and frequency, reminiscent of spontaneous detrusor muscle contractions, and were associated with cell contraction. The majority of these transients reached a magnitude varying from 1/3 to 2/3 of carbachol-induced Ca"^2+" transients. They were Ca"^2+"-dependent and sensitive to the L-type Ca"^2+" channel antagonist verapamil. Activity was also attenuated by blocking SR Ca"^2+" reuptake with thapsigargin. The fraction of spontaneously active cells was 40.2±5.2% in samples from stable bladders (n=44 bladders), and higher in overactive groups (IDO, 73.9±4.8%, n=37, p<0.01; NDO, 73.4±6.7%, n=24, p<0.01). The mean spontaneous increases of [Ca"^2+"], were 12.6±2.3 nM and 14.4±3.8 nM in cells from IDO and NDO patients respectively, compared to 5.7±1.2 nM in cells from stable bladders (p<0.05 for both comparisons). Spontaneous electrical activity in the form of action potentials or depolarising oscillations was also observed, with a higher percentage of cells from IDO bladders (59.2%, n=71 cells vs. 29.5%, n=78 from stable patients, p<0.01) but no significant difference was found in cells from NDO bladders (40.0%, n=20 cells, p>0.05). Further experiments showed that the majority of the depolarising events were accompanied by rises of Ca"^2+", and the magnitude and duration of Ca"^2+" rises also mirrored the membrane depolarising spikes or waves.

Interpretation of results
Unprovoked, autonomous rises of [Ca"^2+"], can be generated in human detrusor smooth muscle cells and mirror spontaneous contractile activity in intact muscle. Their sensitivity to Ca"^2+" antagonists suggests the L-type Ca"^2+" channel and membrane influx constitutes the major source of Ca"^2+" to fuel them, although intracellular stores confer additional modulations. The persistence of activity, its comparable magnitude to muscarinic receptor-mediated responses, as well as association with cell contraction give further credence to its physiological relevance. Altered basal [Ca"^2+"], indicates deranged Ca"^2+" homeostasis in overactive bladders and enhanced [Ca"^2+"], oscillations reveal spontaneous Ca"^2+" activation contributing to upregulated contractile activity. Furthermore, membrane potential oscillations underlie spontaneous Ca rises in IDO but separate mechanisms may contribute to NDO.

Concluding message
Spontaneous, autonomous cellular activity, manifest as [Ca"^2+"], and membrane potential oscillations, originates from detrusor smooth muscle in human bladders. Such cellular activity underlies muscle contraction and upregulated Ca"^2+" activation contributes to increased contractile activity in overactive bladders. Different mechanisms may mediate IDO and NDO.

References
FUNDING: The Wellcome Trust
The Whittington Hospital NHS Trust
HUMAN SUBJECTS: This study was approved by the Joint UCL/UCLH Committees on the Ethics of Human Research and followed the Declaration of Helsinki. Informed consent was obtained from the patients.