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RESEARCH ARTICLE

The oxytocin antagonist cligosiban reduces human prostate contractility: Implications for the treatment of benign prostatic hyperplasia

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[Correction added on 3 May 2024, after first online publication: The copyright has been changed.]

Abstract

Background and Purpose: With increasing life expectancy, benign prostatic hyperplasia (BPH) consequently affects more ageing men, illustrating the urgent need for advancements in BPH therapy. One emerging possibility may be the use of oxytocin antagonists to relax smooth muscle cells in the prostate, similar to the currently used (although often associated with side effects) α_1-adrenoceptor blockers.

Experimental Approach: For the first time we used live-imaging, combined with a novel image analysis method, to investigate the multidirectional contractions of the human prostate and determine their changes in response to oxytocin and the oxytocin antagonists atosiban and cligosiban. Human prostate samples were obtained and compared from patients undergoing prostatectomy due to prostate cancer as well as from patients with transurethral resection of prostate tissue due to severe BPH.

Key Results: The two cohorts of tissue samples showed spontaneous multidirectional contractions, which significantly increased after the addition of oxytocin. Different to atosiban, which showed ambiguous effects of short duration, only long-acting cligosiban reliably prevented, as well as counteracted, any contractile oxytocin effect. Furthermore, cligosiban visibly reduced not only oxytocin-induced contractions, but also showed intrinsic activity to relax prostatic tissue.

Conclusion and implications: Thus, the oxytocin antagonist cligosiban could be an interesting candidate in the search for novel BPH treatment options.

KEYWORDS
clinical pharmacology, muscle contraction, oxytocin receptor, smooth muscle, therapeutics

Abbreviations: AT, atosiban; BPH, Benign prostatic hyperplasia; CL, cligosiban; NT, no treatment; OT, oxytocin; TUR-P, transurethral resection of the prostate.

Beatrix Bester and Kristina Koslow contributed equally to this work and share first authorship.

1 | INTRODUCTION

Benign prostatic hyperplasia (BPH) affects 60% of 60-year-old men with a further age-related increase in prevalence (Berges, 2008). BPH is often accompanied by lower-urinary tract symptoms (Lee & Kuo, 2017). One strategy to alleviate symptoms is to reduce tone and contractility of the prevalent smooth muscle cells of the prostate (Silva et al., 2014). The **α_{1A}-adrenoceptor** is an important mediator for smooth muscle contraction and its inhibition is the first line of medical treatment (Silva et al., 2014). However, treatment with α_1-adrenoceptor blockers often leads to side effects such as ejaculatory disorders (Narayan & Lepor, 2001) and systemic effects such as hypotension. In a previous study, we could show the negative side effects of tamsulosin on seminal vesicles, prostate duct and the sperm-releasing part of the epididymis ex vivo (Seidensticker et al., 2022). Treatment adherence thus is low, ranging from 35% in the first year to only 15% in the fifth year (Cindolo et al., 2015). This amount of discontinuance of treatment, as well as bothersome side effects and an ever-growing increase in population affected, call for new options in the treatment of BPH. The inhibition of other pathways, e.g. the **phosphodiesterase 5 (PDE5)** (Mónica & de Nucci, 2019; Seidensticker et al., 2022), but possibly also **oxytocin** (OT) (Lee et al., 2021) to relax the smooth muscle cells of the prostate was suggested as an alternative therapeutic target. Although oxytocin and the **OT receptor** have been shown to be present in the male reproductive tract (Stadler et al., 2020), the pathway still remains unexploited as a therapeutic target.

As far as we know, there is only one OT receptor antagonist (**atosiban**) that has been approved in Europe (but not in the United States). Although atosiban is regularly used to delay preterm labour (Flenady et al., 2014; Tsatsaris et al., 2004), it has failed to convincingly prove its efficacy in multiple clinical trials (Romero et al., 2000) to delay preterm labour. Interestingly, atosiban has been shown to bind to the arginine-vasopressin receptor 1A (**V_{1A} receptor**) with a higher affinity than to the OT receptor (Manning et al., 2005).

A new, highly selective (>100-fold selectivity for the OT receptor) and potent OT receptor antagonist is cligosiban (Wayman et al., 2018) which is currently undergoing clinical trials for the treatment of premature ejaculation. (Althof et al., 2019; McMahon et al., 2019).

Two studies have investigated the contractile effects of OT in the human prostate (Bodanszky et al., 1992; Lee et al., 2021), both using organ bath. However, the smooth muscle cells in the prostate are interspersed and have a diverse directionality. Therefore, the organ bath may only record the sum of movement along the transducers (thus, some of the responses might be missed or minimized). In contrast to organ bath, live-imaging, also used in male reproductive organs (Mietens et al., 2012; Mietens et al., 2014), including the prostate (Kügler et al., 2018; Seidensticker et al., 2022), detects very small, complex and multidirectional contractions. Most recently, the combination of live-imaging, together with our novel image analysis method allowed the interrogation of movement over the entirety of the tissue, by dividing it into hundreds of regions of interests, quantifying the change in response at each point and comparing them (Stadler et al., 2021).

What is already known?

- Current benign prostatic hyperplasia treatment uses α1-adrenoceptor antagonists to reduce contractility of prostatic smooth muscle.
- α_1-adrenoceptor antagonists have vasculature and ejaculatory side effects, thus targeting other signalling-pathways could be beneficial.

What does this study add?

- Our novel analyses together with live-imaging allowed thorough investigations of the complex human prostatic contractions.
- Oxytocin antagonist cligosiban (but not atosiban) decreased contractions below baseline and counteracted the oxytocin effect.

What is the clinical significance?

- Oxytocin antagonists like cligosiban could develop as alternative therapeutic options for BPH patients.

In this study, to evaluate the feasibility of OT receptor antagonists as a future BPH treatment, we investigated the effect of OT and two OT receptor antagonists (commercial atosiban and one of increasing interest, cligosiban) on human prostatic tissue using live-imaging. We compared, for the first time, samples from transurethral resection of the prostate (TUR-P) (Portis & Mador, 1997) to samples from radical prostatectomy.

2 | METHODS

2.1 | Study approval

Human prostate tissue was collected from informed patients of the University's Hospital of Giessen (Clinic for Urology, Paediatric Urology and Andrology) with the approval of the Ethics Committee of the Medical Faculty of the Justus-Liebig-University Giessen (AZ 123/12; 55/13) conforming to ethical standards. Tissue samples were obtained from patients undergoing radical prostatectomy, due to prostate cancer, or transurethral resection of the prostate (TUR-P), due to benign prostatic hyperplasia. To minimize personal contact during the corona pandemic with particularly vulnerable cancer patients, we were unable to obtain any more prostatectomy samples for the experiments using cligosiban and continued our experiments with the TUR-P samples only.

2.2 | Study design

Each experiment started with a no treatment (NT) period to observe spontaneous contractility and ended with a vitality check to confirm tissue responsiveness (graphical study design in Figure S1). The vitality check was performed with 10-μM NA (noradrenaline or noradrenaline bitartrate salt) (Sigma-Aldrich, Steinheim, Germany) or 80 mM KCl (potassium chloride) (Merck KGaA, Darmstadt, Germany) after noticing that the response to NA was unsatisfactory in some TUR-P samples (probably due to the long-term BPH treatment of TUR-P patients with α1-adrenoceptor blockers, see Section 4).

In a first round of experiments to test the effect of OT on the contractility of the human prostate, 0.5-μM OT (oxytocin acetate salt) (Bachem, Bubendorf, Switzerland) was added after the NT period with $n = 6$ for both cohorts of samples from prostatectomy as well as TUR-P patients.

To test and compare the capabilities of two different OT antagonists (atosiban and cligosiban) in blocking the previously seen OT effect, the substances were added either (i) before or (ii) after the OT period (randomized) in successive experiments (Figure S1) using neighbouring samples from the same patient. First, a total of 19 experiments with TUR-P samples and 13 with prostatectomy samples were performed using 2-mM atosiban (AT) (Sigma-Aldrich, Steinheim, Germany). Second, a total of 19 experiments with TUR-P samples were performed using 40-μM cligosiban (CL) (MedChemExpress, Monmouth Junction, NJ, USA). As such, randomisation of atosiban versus cligosiban application was not performed as cligosiban experiments were only started after sample collection for atosiban experiments were completed.

The concentrations for all agents including OT and the OT receptor antagonists were based on our previously published results on their effects in the epididymis (Stadler et al., 2021) aiming to completely counteract/prevent OT-induced contractions as well as evaluate any effect on spontaneous contractility. To achieve this we had determined that at least 5 μM of atosiban and at least 40 μM of cligosiban was necessary to prevent the strong OT effect otherwise observed in this tissue. With 2.5-μM atosiban, OT still showed a significant effect while the OT effect was abolished with 5-μM atosiban in the epididymal tissue (Figure S2a). Only with 40-μM cligosiban, the OT effect was abolished, whereas 20 μM and notably 10 μM of cligosiban still allowed a significant OT effect in the epididymal tissue (Figure S2b). In addition, four increasing concentrations (2, 20, 200 μM and 2 mM) of atosiban were compared to the strong contractions occurring spontaneously (NT) in the rat prostate (Figure S2c). Even with the highest concentration of 2-mM atosiban, contractions could not be fully inhibited. Since we wanted to display the maximum relaxing effect achievable, we thus decided to use the highest concentration (2 mM) of atosiban and 40-μM cligosiban in human prostate samples for this study.

Tissue was only included in the analysis if it was responsive to the vitality check. In case of the antagonist experiments, samples were excluded if only one of the two neighbouring samples was responsive to the vitality check/usable. All collected samples and the resulting n-numbers for analysis are summarized in tables and can be found in the supplementary data (Figure S3). For further explanation on tissue n-numbers, please see Section 2.5.

2.3 | Tissue preparation

In case of prostatectomy, human prostate samples were obtained from cancer-free areas and as such a specific zonation could not be followed. In case of TUR-P, 5–10 deep strips of maximum length (usually 2–5 cm) were quickly excised directly at the beginning of the procedure at 5 and 7 o'clock positions proximal to the colliculus seminalis (between bladder and colliculus seminalis) at a safe distance from the bladder sphincter using a monopolar technique with a fully immersed sling. This allowed for as little coagulation and heat development as possible in the area of the incisions for the tissue samples. As such, the tissue samples of TUR-P patients represent a cohort of similar sample location, probably from the transition zone. After removal, the tissue was immediately placed in Minimal Essential Medium (MEM; Thermo Fisher Scientific, Waltham, MA, USA) and prepared for the experiments within the next 2 hours. Small strips (max. $5 \times 0.5 \times 0.5$ mm [length $\times$ width $\times$ thickness]) were prepared from the human prostate samples. In case of the TUR-P samples the coagulated outer tissue had to be removed first to make sure to use the most viable tissue in the sample.

2.4 | Live-imaging

For live-imaging, rat collagen was used as embedding material, as described by Mietens et al. (2014). Live-imaging experiments were performed on a Leica DM5000 B microscope using an ORCA-Flash4.0 Hamamatsu digital camera and LAS X software. Dishes were positioned under transmitted light and kept at 33°C at all times. Images were taken with a 10X magnification at a time interval of 2 s.

2.5 | Data and statistical analysis

Data and statistical analysis complied with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology (Curtis et al., 2022). We aimed for the group sizes to be the same in prostatectomy and TUR-P cohorts as well as for the comparison of atosiban and cligosiban. All collected tissue samples with their respective responsiveness assessment and decision on inclusion or exclusion are summarized in a table (Figure S3). For a first set of experiments ('no treatment' followed by the addition of oxytocin) with the respective two prostate tissue samples groups (prostatectomy and TUR-P), we collected six samples each. Based on the results from the first TUR-P experiments we set 0.2 as an upper bound for the true standard deviation of differences and 0.15 as the

least practically relevant true difference in means. Based on the results from the first prostatectomy experiments we set 1.5 as an upper limit for the true standard deviation of differences and 1.1 as the least practically relevant true difference in means. For both groups, we estimated the required sample size for Student's one-sided paired two-sample t-test with a power of 80% and a significance level of 5%, and obtained for both groups a required sample size of $n = 13$ for the planned antagonist experiments. In addition, we gathered from our first round of experiments that a fraction of TUR-P samples would be lost for statistical analysis (2 out of 6). Based on this expected loss of 1/3, we collected an excess of TUR-P samples to ensure similar group sizes for the comparison with prostatectomy samples. Thus, 19 TUR-P samples were collected for atosiban and cligosiban experiments each.

In case of the atosiban experiments, 11 of 19 collected samples had to be excluded based on tissue responsiveness. One additional sample only showed responsiveness in one of the two set-ups and was thus excluded as well. This resulted in $n = 7$ for the TUR-P samples included in statistical analysis for atosiban experiments.

In case of the cligosiban experiments, 9 of the 19 collected samples had to be excluded based on tissue responsiveness. Three additional samples only showed responsiveness in one of the two set-ups and were thus excluded as well. This resulted in $n = 7$ for the TUR-P samples included in statistical analysis for cligosiban experiments.

In case of samples collected from prostatectomy patients, all 13 samples collected showed tissue responsiveness. However, we had technical issues with the live-imaging set-up in the two last samples collected and thus although the samples were responsive we could not include them in the statistical analysis.

As such, we ended up with seven TUR-P samples each and 11 prostatectomy samples for the oxytocin receptor antagonist experiments. The n numbers reported are independent values and statistical analysis was performed based on these independent values.

Image processing, analysis and montage of the images into short films were performed using Fiji (ImageJ) (Schindelin et al., 2012). We used a further development of the Wiggle Index (Denecke et al., 2015; Preston et al., 2015, 2016) first validated in the epididymis (Stadler et al., 2021), which allowed us to quantify and compare the sum of movements of the entirety of the tissue by dividing it into hundreds of regions of interests. The live-imaging data was analysed by this custom automated Fiji macro (Schindelin et al., 2012) and as such excluded the possibility for human bias. Therefore, no blinding of the data was necessary. To reduce noise and artefacts in the videos (such as debris and small movements due to liquid vibration) a Gaussian Blur was applied to each image sequence as well as the original image. Additionally, to limit false positive movement scores the images were then binned into a 4×4 grid, that is, a pixel array of 4×4 pixels were combined into one super pixel with the final value being the average of the original intensity of the 16 pixels. The standard deviation of the intensity of each of these pixels was calculated over a window of 30 frames. This window was then shifted one frame forward and calculated again. This resulted in an image stack with each super pixel representing the rolling average of the standard deviation of each super pixel. This rolling average stack was then used to calculate the final movement score for a given super pixel, which represents the average of the rolling average standard deviation.

Data were logged per super pixel movement score and displayed as a heat map with each pixel representing the assigned movement score. The per pixel data allowed graphing and statistical analysis of the pooled data for each time period from each data set. For comparison between different experiments, the relative data was baseline corrected by expressing the movement values of drug treatment segments as a relative change to its equivalent baseline pixel. The heat map images allow the visualization of where a given movement score occurs within the tissue (Figure S4).

Data are shown on the graphs as cumulative frequency distributions ± standard deviation (±SD). In this manner, data were sorted by increasing movement score from left to right by adding up to 100% of data points (1.0 in fractions as seen in the graphs). The distribution of data in groups determined by a calculated bin size (movement score) refers to the points on the rising lines on the graph. In this fashion, groups with higher movement scores (more movement) will be 'added' in the cumulative graph only further to the right. This means that for data sets with no groups with higher movement scores, the line of the graph will rise up and plateau at 1.0 much quicker than in a data set with many higher scores (Figure S4).

Statistical analysis of the movement scores was performed by using non-linear regression of the cumulative frequency distributions of the scores by comparing the curve fits of each distribution. If one fit could not adequately explain each graph they were classified as significant, with a p-value assigned ($P < 0.05$ was accepted as significant). No outliers were defined or excluded from analysis. GraphPad (GraphPad Prism 9, version for Windows, GraphPad Software, La Jolla, California, USA, www.graphpad.com) was used to create all graphs and run statistical analysis.

The NT, OT, AT and CL periods were each analysed in the beginning (NT1, OT1, AT1 and CL1) as well as in the end (NT2, OT2, AT2 and CL2) of each recording period to adequately detect true changes to the addition of the substances and exclude gradual changes of contractility over time. Therefore, usually the end of one period was compared to the beginning of the subsequent period.

In addition, the specific spatial information visualized in the heat maps allowed us to determine and display exactly which specific areas of tissue moved relative to the rest of the tissue.

2.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander, Christopoulos et al., 2023; Alexander, Fabbro et al., 2023).

3 | RESULTS

3.1 | Oxytocin (OT)

The OT effect was analysed by comparing the no treatment (NT) period just before addition of OT (NT2) to the period 1 min after addition of OT (OT1) (Figure 1a), as OT shows a rapid onset of action (Gimpl & Fahrenholz, 2001). Human prostate tissue samples contracted spontaneously in the NT period. OT had a significant increasing effect on those spontaneously occurring contractions of the human prostate samples (Figure 1b,c).

Samples originated from two different surgical procedures, TUR-P samples from patients with BPH and radical prostatectomy samples from patients with prostate carcinoma. It seemed essential to include tissue samples originating from TUR-P since these patients presented with clinically severe symptoms of BPH and as such would be the target group for novel medical BPH-treatment. Prostatectomy patients who presented for prostate cancer were usually neither medicated for BPH nor showed clinically relevant BPH-symptoms when admitted for surgery. Prostatectomy samples therefore could represent untreated, 'healthier' prostate tissue and as such could show a different responsiveness to substances compared to TUR-P samples (independent of the differing operation technique, zonation and tissue responsiveness). Therefore, we also evaluated the two cohorts separately to highlight their potential differences. The significant increasing OT effect could be shown in both tissue sample cohorts separately (Figure 1d): prostatectomy and TUR-P respectively.

It was noted that (i) the overall spontaneous contractility as well as (ii) the stimulating effect of OT were significantly more pronounced in samples originating from prostatectomy than from TUR-P (Figure 2). Additionally, we wanted to know if the examined spontaneous contractility in the no treatment (NT) period and the subsequent change in contractility after OT addition (OT1) correlated with our collected patient data. These parameters from both surgical procedures included: age, prostate specific antigen (PSA) level, prostate volume and when possible International Prostate Symptom Score (IPSS). (Figure S5a prostatectomy and Figure S6a TUR-P respectively).

In prostatectomy samples, we found that age correlated positively with PSA level (Figure S5B) and prostate volume (Figure S5c), which has been described in the literature (Chung et al., 2006; Luboldt et al., 2007). For prostatectomy samples, no other correlations could be identified.

In TUR-P samples, PSA level correlated positively with prostate volume (Figure S6b). This has also already been described in the literature (Chung et al., 2006). Another (positive) correlation in TUR-P samples was found to be between the contractility in the NT section and the contractility after OT addition (Figure S6c). This could be attributed to the fact that TUR-P tissue was always obtained from the same prostate zone and thus showed similar contractility, while prostatectomy samples were obtained from different regions (i.e., outside the tumour). For TUR-P samples, no other correlations could be identified.

3.2 | Atosiban (AT)

Having established an OT effect in human prostatic tissue, we turned our attention to testing OT receptor antagonists, with the intention to use them as possible therapeutic options for BPH in the future. First, we investigated atosiban, a commercial OT receptor antagonist, which had been used in clinical practice for over two decades to delay preterm labour. In both the prostatectomy and TUR-P samples, atosiban was added either before or after OT treatment.

In OT-treated prostatic tissue (Figure 3) atosiban had a significant decreasing effect on the contractility of prostate samples acquired through prostatectomy (Figure 3c) (for video, see Video S7) but not on the ones acquired through TUR-P (Figure 3d). (All observed substance effects compared to the maximum effect induced by noradrenaline can be seen as supporting information; Figure S8.) Overall, atosiban showed ambiguous effects: While 9 out of 11 prostatectomy samples and 5 out of 7 TUR-P samples showed the expected decrease in contractility, which was significant in case of the prostatectomy samples, an increase in contractions was observed after atosiban addition in the other samples (too little for statistical analysis). The differences in the effect of atosiban can also be clearly seen in the corresponding heat maps and tables (Figure 3b–d).

Comparable atosiban effects were found without prior OT treatment, i.e. decreasing as well as increasing effects (Figure 4). Although a tendency towards an increased contractility could be observed in the graphs, atosiban had no significant effect on previously untreated tissue (prostatectomy [Figure 4c] and TUR-P [-Figure 4d]). Even when analysing the groups with decreasing or increasing effects separately, atosiban still had no significant effect on spontaneous contractility.

To test the capability of atosiban to prevent OT action, the effect of OT was evaluated in prostatic tissue that had been pre-treated with atosiban (Figure 5). OT did not have a significant effect in the TUR-P samples (Figure 5d). However, in the prostatectomy samples, a significant effect of OT was still recorded (Figure 5c).

In prostatectomy specimens, the relative change of contractility between no treatment (NT2) and OT (OT1) was compared with or without atosiban treatment prior to OT addition. A significantly reduced OT effect could be observed when the tissue had been pre-treated with atosiban (Figure 6). This suggests that atosiban could reduce OT-induced contractions (seen in Figure 5) but did not completely block contractions.

In contrast to the no treatment and OT period, where the contractions seemed stable over time (not significantly different between the beginning and the end of one period), contractions during the atosiban period did not seem stable. There was a significant increase in contractility from the beginning (AT1) towards the end (AT2) of the atosiban period (Figure S9) suggesting a short duration of action of atosiban which might be an explanation for the lack of a complete blocking effect in Figure 5.

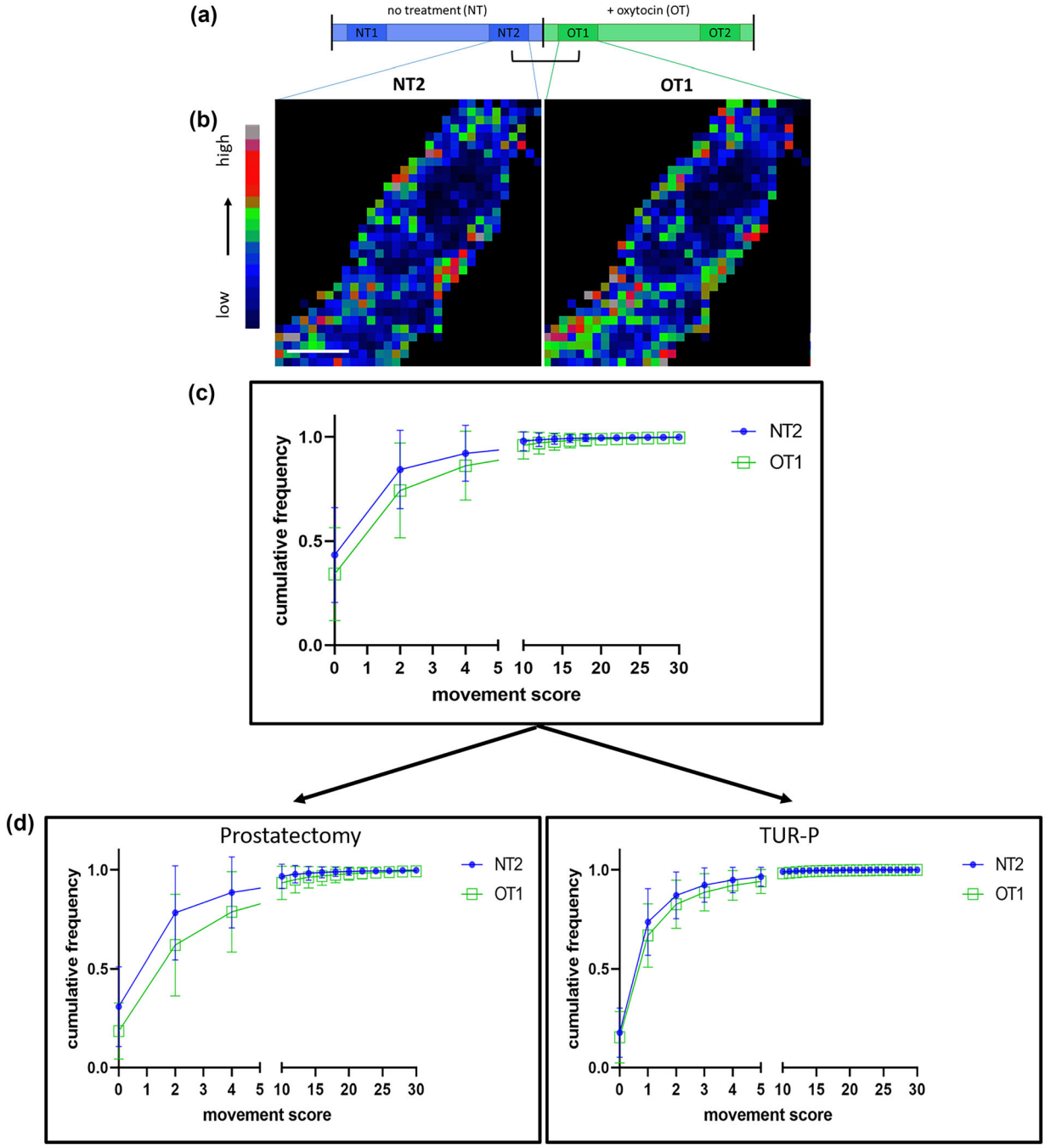


FIGURE 1 The effects of oxytocin on human prostate tissue originating from prostatectomy and TUR-P. For information about group sizes and loss of numbers, please refer to Section 2. (a) Schematic of experimental setup: comparison between no treatment (NT2) and oxytocin treatment (OT1). (b) Heat map representation of movements in the two analysed sections (NT2 and OT1) (white scale bar: 300 μm). (c) Graph of the cumulative frequency distribution (±SD) comparing NT2 and OT1. Oxytocin significantly increased contractility compared to the no treatment period (n = 35) ($P < 0.0001$). (d) Left: Graph of the cumulative frequency distribution (±SD) comparing NT2 and OT1 in prostatectomy samples. Oxytocin significantly increased contractility (n = 17) ($P < 0.0001$). Right: Graph of the cumulative frequency distribution (±SD) comparing NT2 and OT1 in TUR-P samples. Oxytocin significantly increased contractility compared to the no treatment period (n = 18) ($P < 0.0001$).

3.3 | Cligosiban (CL)

As atosiban often displayed variable effects, we investigated the novel, highly selective, potent OT receptor antagonist cligosiban. For these experiments, only TUR-P samples were available to us. In contrast to the prompt-acting but only short-lasting atosiban, cligosiban's effect was time dependent, visible in the significant decrease in contractility of the OT-treated tissue between the beginning (CL1) and

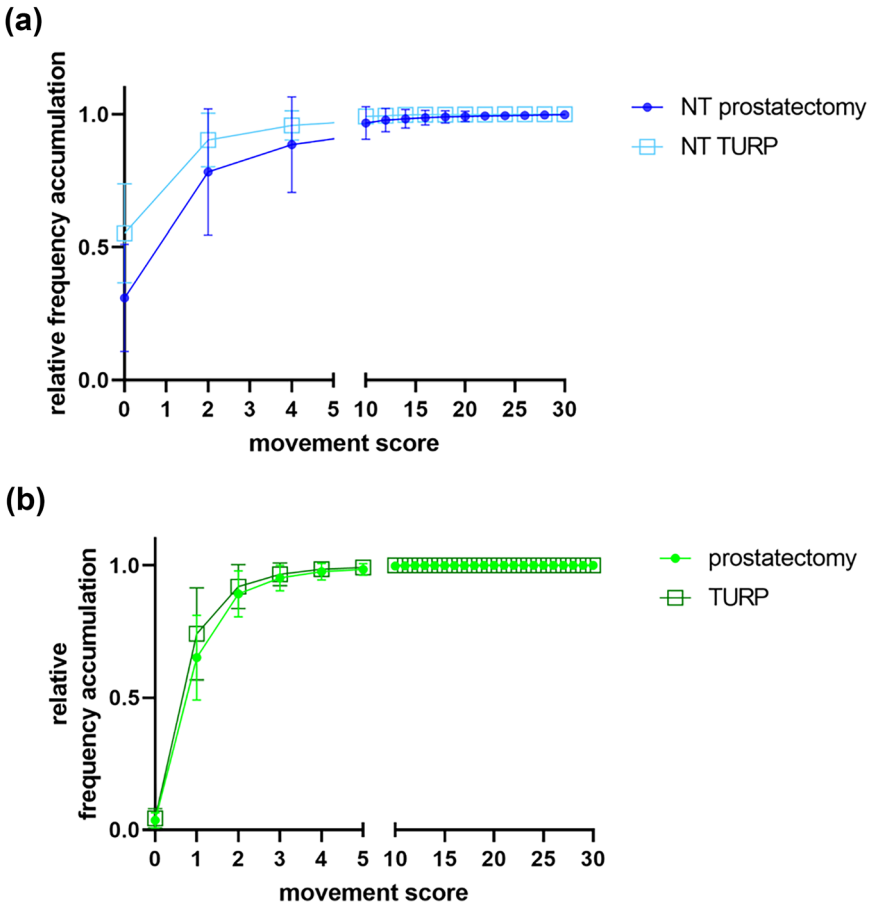


FIGURE 2 Comparison between prostatectomy and TUR-P. For information about group sizes and loss of numbers, please refer to Section 2. (a) Graph of the relative frequency accumulation (±SD): Samples from prostatectomy showed significantly more spontaneous contractility than samples from TUR-P in the no treatment period ($P < 0.0001$). (b) Graph of the relative frequency accumulation (±SD): Samples from prostatectomy significantly increased more in contractility after the addition of oxytocin than samples from TUR-P ($P < 0.0001$).

the end (CL2) of the cligosiban period (Figure S10). Therefore, we used the end of the cligosiban period (CL2) for the following comparisons.

Cligosiban (CL2, end of period) had a significant decreasing effect on the contractility of the OT-treated prostate samples (Figure 7) (for video, see Video S11). This effect was not significant at the beginning of the cligosiban period (CL1) (Figure S12). (All observed substance effects compared to the maximum effect induced by potassium chloride can be seen as supporting information; Figure S13.) Interestingly, contractions of the OT-treated tissue during the CL2 period were also significantly decreased in comparison to the no treatment period (NT2) before OT addition (Figure 8). This suggests that cligosiban not only counteracted the OT effect, but even decreased contractions below baseline contractility.

To test the capability of cligosiban to prevent OT action, the effect of OT was evaluated in prostatic tissue that had been pre-treated with cligosiban (Figure 9). OT did not have a significant effect (Figure 9c) in cligosiban-treated prostatic tissue. For future clinical application, it is of interest if cligosiban would be effective without exogenous addition of OT. In most TUR-P tissue samples a clear decreasing trend of cligosiban (CL2) in comparison to no treatment (NT2) was visible but could not reach significance in our cohort (Figure 10).

4 | DISCUSSION

Our live-imaging results from this and previous studies (Kügler et al., 2018) using human prostate samples confirm that there are spontaneous contractions in the human prostate without a pre-tension on the tissue, as it had to be done in all previous studies using oxytocin in organ baths (Bodanszky et al., 1992; Gupta et al., 2008; Lee et al., 2021; Sharaf et al., 1992). Our multidirectional contractions increased after the addition of oxytocin in both cohorts of prostatic samples originating from either prostatectomy or TUR-P. The prostatectomy samples were significantly more contractile during the no treatment period and increased more after the addition of oxytocin compared to TUR-P samples. This could be due to the tissue from TUR-P patients being more damaged because of the extraction process and therefore less responsive. Another reason could be that most of the TUR-P patients from whom our samples originated received medical treatment for BPH, even on the day of surgery (Tamalunas et al., 2021). Interestingly, a study found that after long-term-treatment with tamsulosin, concentrations of free as well as bound tamsulosin in the prostate tissue could still be shown 4, 8, 24 and even 48 hours after the last tablet had been taken (Korstanje et al., 2011). As we ran our experiments directly after we received the tissue from surgery, it seems likely that there was still tissue-bound tamsulosin, which might still have exerted its relaxing and blocking

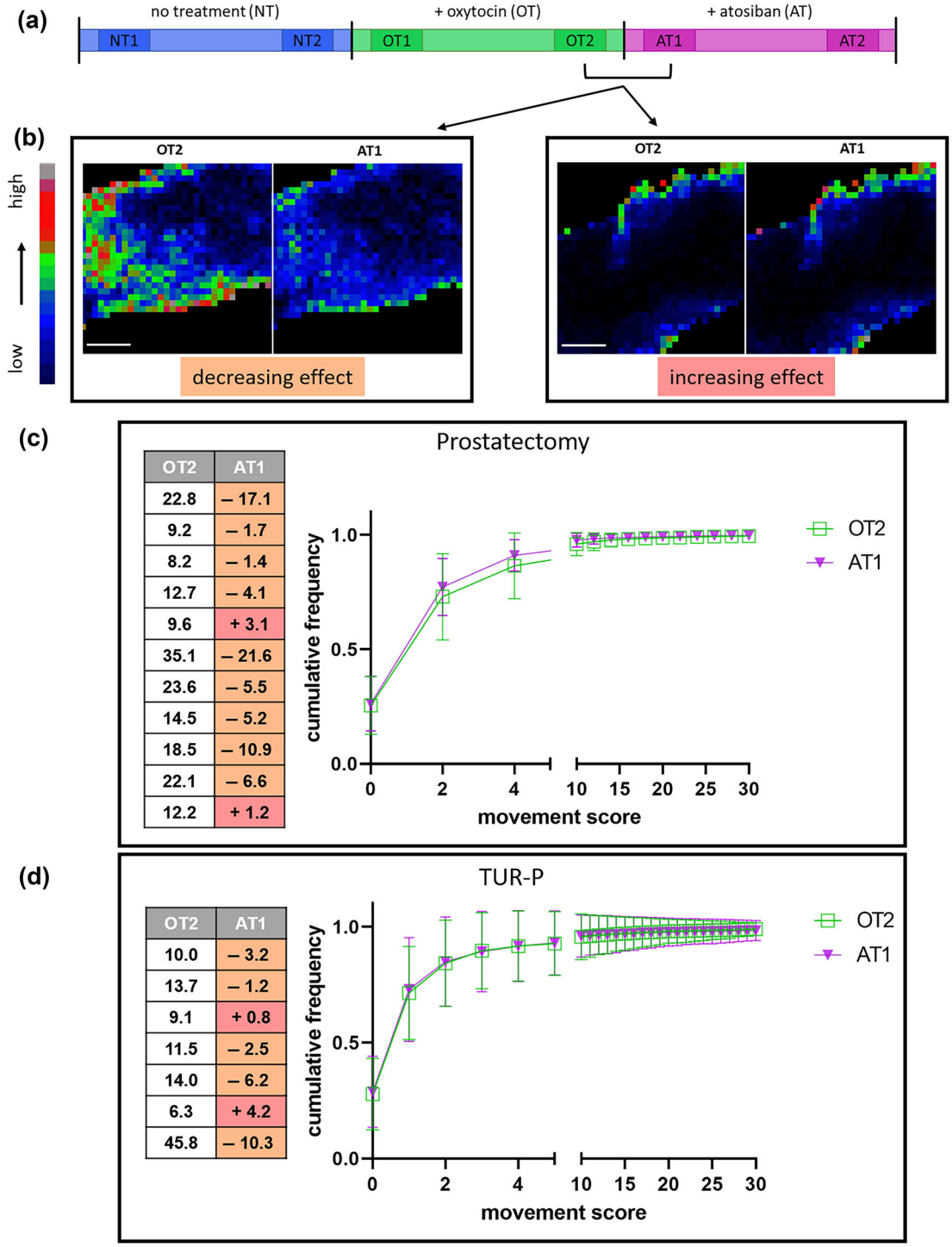
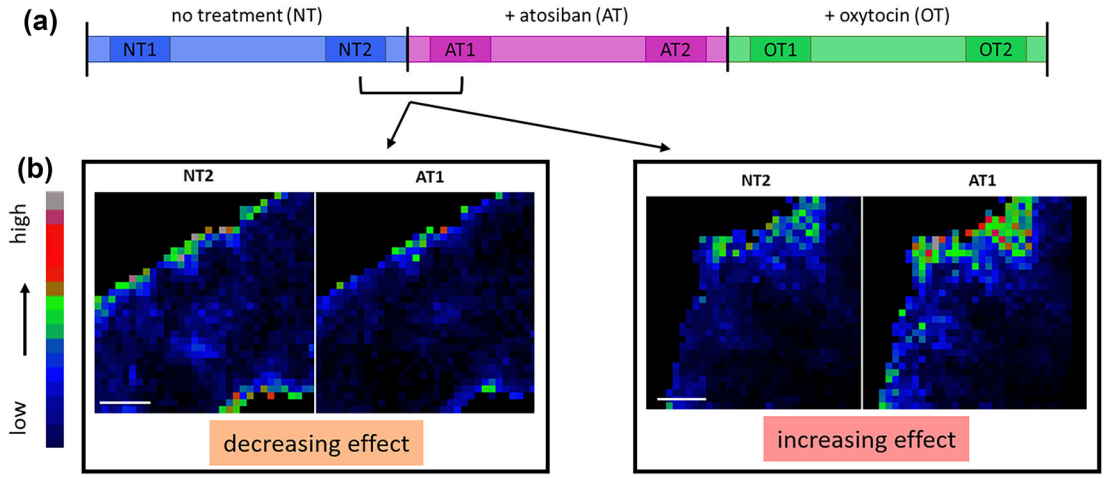
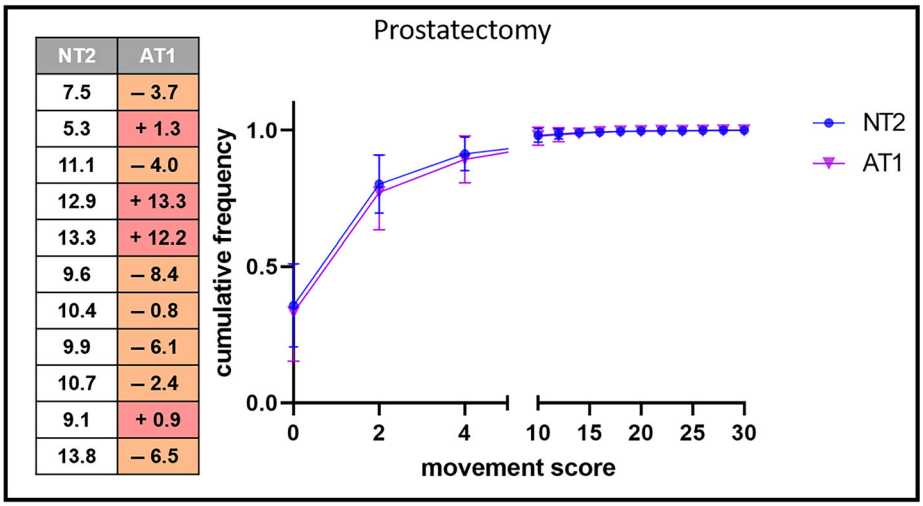


FIGURE 3 The effects of atosiban after oxytocin treatment on human prostate tissue originating from prostatectomy and TUR-P. For information about group sizes and loss of numbers, please refer to Section 2. (a) Schematic of experimental setup: comparison between oxytocin treatment (OT2) and atosiban treatment (AT1). (b) Heat map representation of movements in the two analysed sections (OT2 and AT1) (white scale bar: 300 μm). The left heat maps show the decreasing effect, and the right heat maps the increasing effect of atosiban. (c) Left: Table showing the percentage of all movements greater than 2 standard deviations (SD > 2) within the OT2 section and the corresponding change of SD > 2 after subsequent atosiban treatment (AT1) in prostatectomy samples, colour-coded into decreasing and increasing values. Right: Graph of cumulative frequency distribution (±SD) comparing OT2 and AT1. Atosiban significantly decreased contractility compared to the oxytocin period (n = 11) ($P = 0.0441$). Increasing (n = 2, too little for statistics) as well as decreasing (n = 9, $P = 0.0107$) effects were noticed. (d) Left: Table showing the percentage of all movements greater than 2 standard deviations (SD > 2) within the OT2 section and the corresponding change of SD > 2 after subsequent atosiban treatment (AT1) in TUR-P samples, colour-coded into decreasing and increasing values. Right: Graph of cumulative frequency distribution (±SD) comparing OT2 and AT1. Atosiban showed no significant effect (n = 7) ($P \geq 0.05$). Increasing (n = 2, too little for statistics) as well as decreasing (n = 5, $P \geq 0.05$) effects were noticed.



(c)

NT2	AT1
7.5	– 3.7
5.3	+ 1.3
11.1	– 4.0
12.9	+ 13.3
13.3	+ 12.2
9.6	– 8.4
10.4	– 0.8
9.9	– 6.1
10.7	– 2.4
9.1	+ 0.9
13.8	– 6.5



(d)

NT2	AT1
8.3	+ 0.3
7.9	– 0.8
10.7	– 4.0
11.2	+ 2.5
6.3	+ 16.1
6.9	– 4.3
14.0	– 3.3

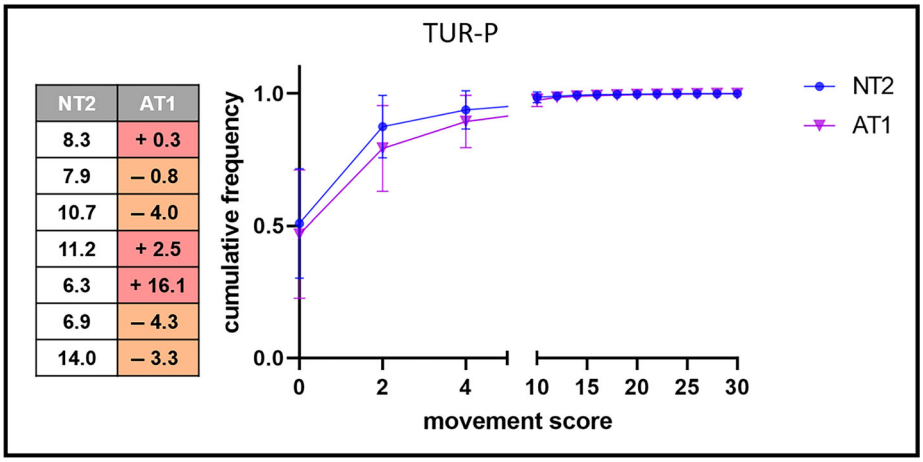


FIGURE 4 The effects of atosiban compared to no treatment on human prostate tissue originating from prostatectomy and TUR-P. For information about group sizes and loss of numbers, please refer to Section 2. (a) Schematic of experimental setup: comparison between no treatment (NT2) and atosiban treatment (AT1). (b) Heat map representation of movements in the two analysed sections (OT2 and AT1) (white scale bar: 300 μm). The left heat maps show the decreasing effect and the right heat maps the increasing effect of atosiban. (c) Left: Table showing the percentage of all movements greater than 2 standard deviations (SD > 2) within the NT2 section and the corresponding change of SD > 2 after following atosiban treatment (AT1) in prostatectomy samples, colour-coded into decreasing and increasing values. Right: Graph of the cumulative frequency distribution (±SD) comparing OT2 and AT1. Atosiban showed no significant effect (n = 11) ($P \geq 0.05$). Increasing (n = 4, too little for statistics) as well as decreasing (n = 7, $P \geq 0.05$) effects were noticed. (d) Left: Table showing the percentage of all movements greater than 2 standard deviations (SD > 2) within the NT2 section and the corresponding change of SD > 2 after following atosiban treatment (AT1) in TUR-P samples, colour-coded into decreasing and increasing values. Right: Graph of the cumulative frequency distribution (±SD) comparing NT2 and AT1. Atosiban showed no significant effect (n = 7) ($P \geq 0.05$). Increasing (n = 3, too little for statistics) as well as decreasing (n = 4, too little for statistics) effects were noticed.

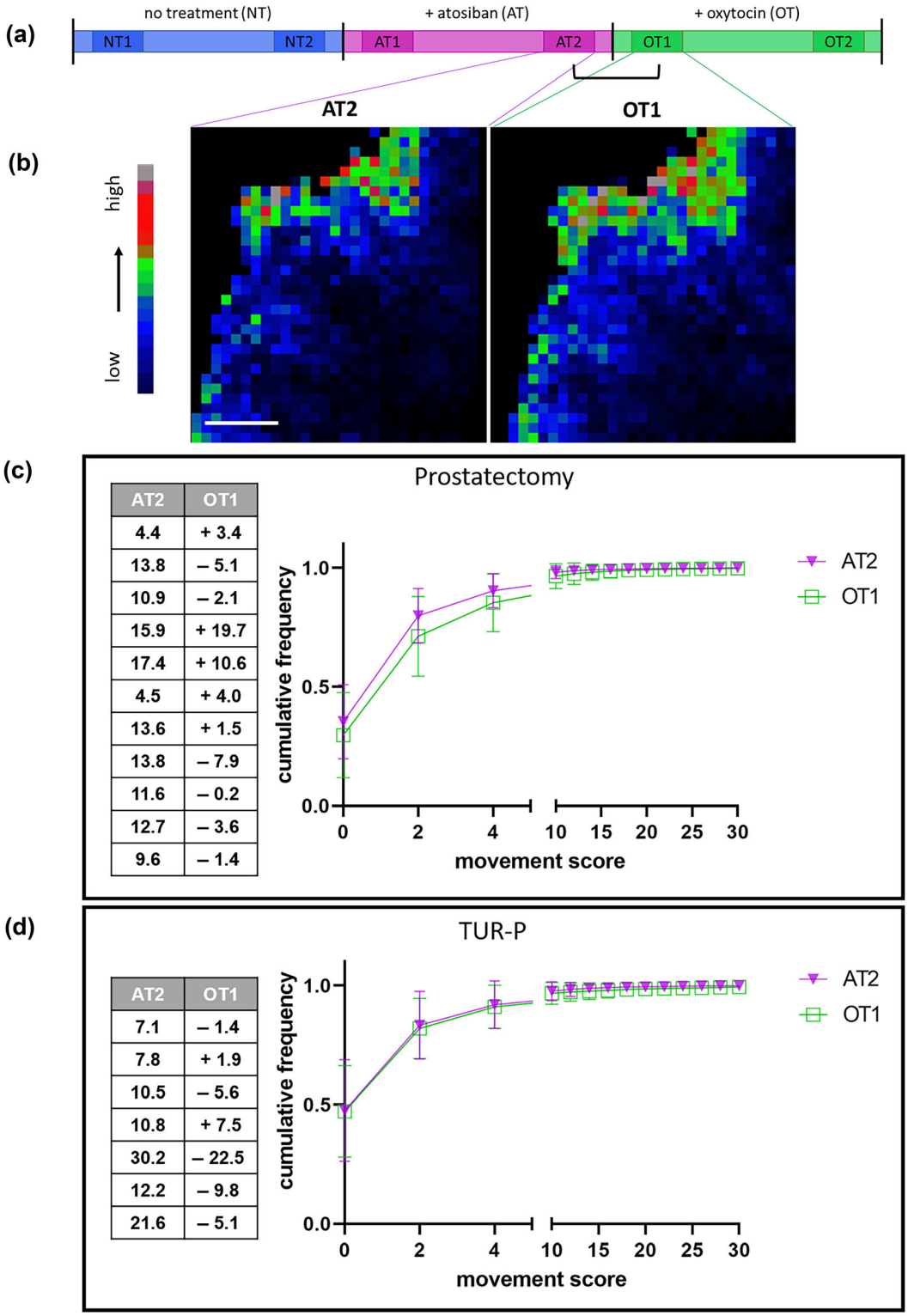


FIGURE 5 The effect of oxytocin after previous atosiban treatment on human prostate tissue originating from prostatectomy and TUR-P. For information about group sizes and loss of numbers, please refer to Section 2. (a) Schematic of experimental setup: comparison between atosiban treatment (AT2) and oxytocin treatment (OT1). (b) Heat map representation of movements in the two analysed sections (AT2 and OT1) (white scale bar: 300 μm). (c) Left: table showing the percentage of all movements greater than 2 standard deviations (SD > 2) within the AT2 section and the corresponding change of SD > 2 after following oxytocin treatment (OT1) in prostatectomy samples. Right: Graph of the cumulative frequency distribution (±SD) comparing AT2 and OT1. Oxytocin showed a significant effect (n = 11) ($P = 0.0016$). (d) Left: Table showing the percentage of all movements greater than 2 standard deviations (SD > 2) within the AT2 section and the corresponding change of SD > 2 after following oxytocin treatment (OT1) in TUR-P samples. Right: Graph of the cumulative frequency distribution (±SD) comparing AT2 and OT1. Oxytocin showed no significant effect (n = 7) ($P \geq 0.05$).

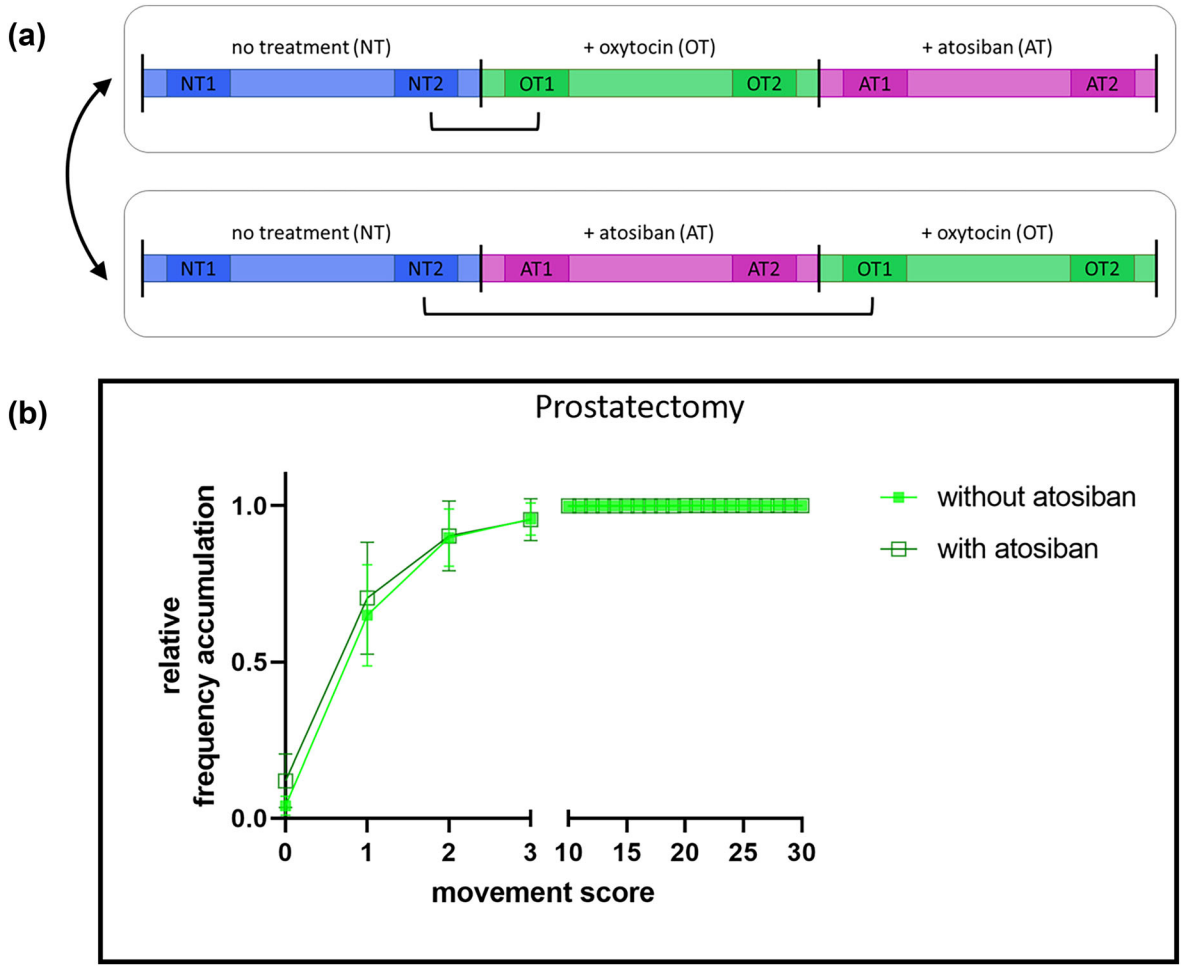


FIGURE 6 Comparison of the relative effects of oxytocin to no treatment with or without pre-treatment of atosiban. For information about group sizes and loss of numbers, please refer to Section 2. (a) Schematic of experimental setup: Comparison between the relative change from no treatment (NT2) to oxytocin treatment (OT1), with and without pre-treatment with atosiban (AT). (b) Graph of the cumulative relative frequency distribution (±SD) of the fold change after oxytocin addition between setups with and without pre-treatment of atosiban (n = 11) ($P = 0.0070$).

abilities ex vivo. This could explain why our tissue samples from TUR-P patients did not respond to noradrenaline while all samples from prostatectomy patients (no BPH pre-medication) responded well. The difference in contractility between prostatectomy and TUR-P samples could also be due to being obtained from different prostatic zones.

Our results demonstrate that TUR-P tissue, although less contractile than prostatectomy samples, can be used to investigate the effect of different agents on the contractility of the human prostate and might mimic the diseased BPH prostate more closely.

For the no treatment (NT) as well as the OT period, we found no significant difference between the contractility at the beginning versus the end of the observation period. In contrast, for the two antagonists we used, we observed a significant difference between the start and the end of the observed period.

Furthermore, when evaluating the two antagonists for their ability to block the action of oxytocin, we found that only cligosiban, not atosiban, reliably prevented any action of oxytocin. We also observed a difference of action between the two oxytocin receptor antagonists in our experiments. Atosiban was fast-acting (immediate full effect) but only for a short period of time (2–4 min), while cligosiban reached its full antagonizing effect only after 3–5 min, which then lasted until the end of the experiments (minimum of 15 min). One reason for this observed difference in action could be that atosiban and cligosiban structurally differ from one another: atosiban is a (nona) peptide and cligosiban a non-peptide. Their respective pharmacodynamics could therefore vary, including receptor binding and competition with oxytocin. For both atosiban and cligosiban, it has been shown that their antagonistic effect at the oxytocin receptor was reversible (Reversi et al., 2005; Wayman et al., 2018).

Interestingly, we could observe that atosiban in fact had an ambiguous effect. Rather than reliably acting as an antagonist for which it is certified to be used in Europe to prevent preterm labour, it sometimes had an effect to increase contractions. This might be why atosiban has been found ineffective in preventing preterm labour in some clinical trials (Romero et al., 2000) and is not certified as a treatment for preterm labour in the United States of America. In addition

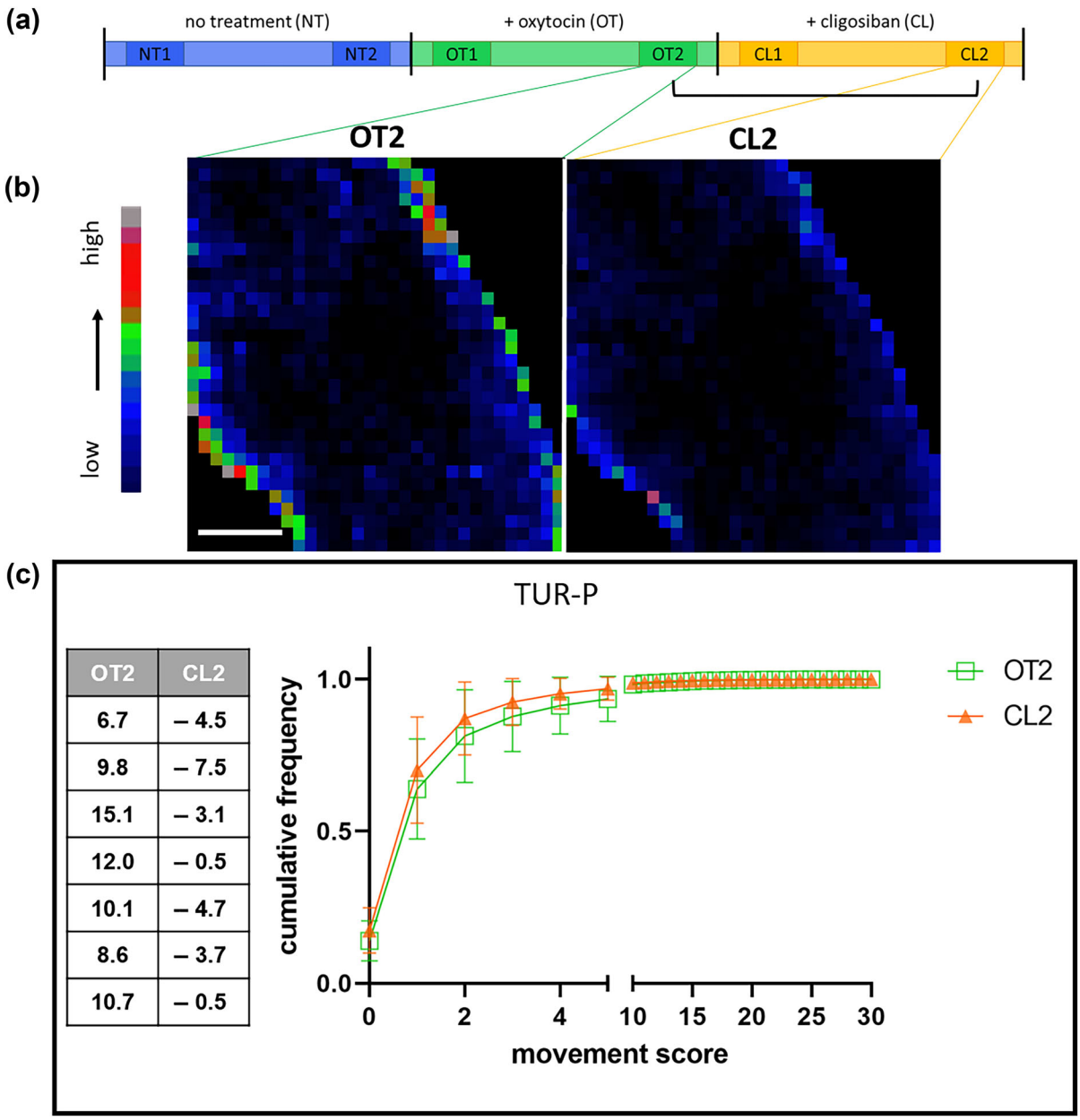


FIGURE 7 The effect of cligosiban after oxytocin treatment on human prostate tissue originating from TUR-P. For information about group sizes and loss of numbers, please refer to Section 2. (a) Schematic of experimental setup: comparison between oxytocin treatment (OT2) and cligosiban treatment (CL2). (b) Heat map representation of movements in the two analysed sections (OT2 and CL2) (white scale bar: 300 µm). (c) Left: Table showing the percentage of all movements greater than 2 standard deviations (SD > 2) within the OT2 section and the corresponding change of SD > 2 after following cligosiban treatment (CL2). Right: Graph of the cumulative frequency distribution (±SD) comparing OT2 and CL2. Cligosiban significantly decreased contractility compared to the oxytocin period (n = 7) ($P < 0.0001$).

to acting as an antagonist on the oxytocin receptor with its downstream signalling through the Gq-subunit protein, Reversi and colleagues suggested for atosiban an additional possible downstream signalling involving the Gi-subunit (Reversi et al., 2005). This Gi-subunit is usually associated with anti-proliferative effects (Lerman et al., 2018) but could also activate phospholipase C beta and therefore an influence on contractility cannot be excluded as well. Based on this, it is thinkable that the observed decreasing effect of atosiban on contractility results from competitive binding to the oxytocin receptor (signalling through the Gq-subunit) while the increasing effect could be explained by activating oxytocin receptors signalling through a Gi-subunit. In view of the high atosiban concentration used in our experiments, such off-target effects could be possible.

In contrast, cligosiban not only reliably blocked oxytocin in the human prostate samples, but it also visibly reduced contractions compared to the no treatment period.

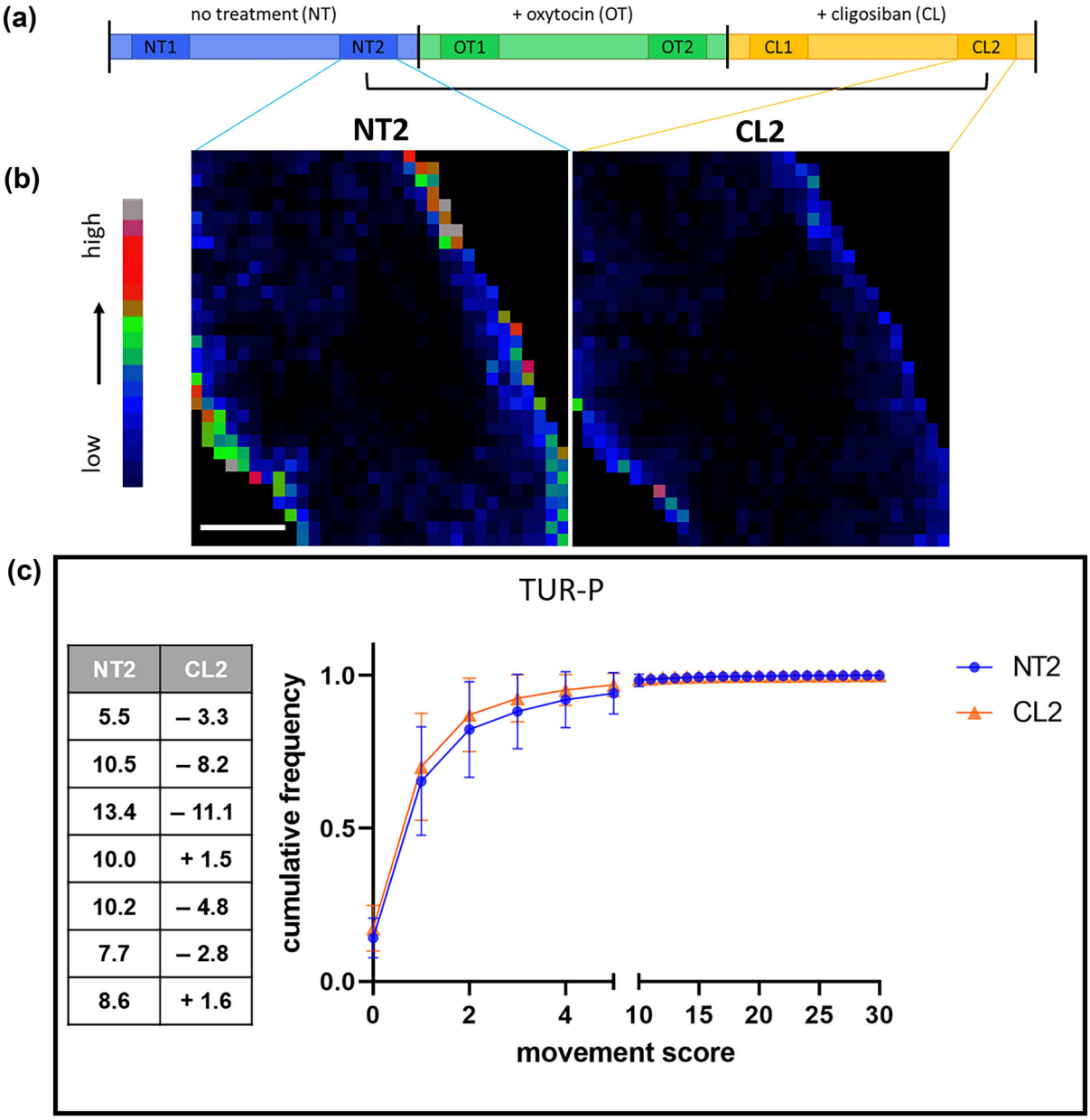


FIGURE 8 The effect of cligosiban compared to no treatment after previous oxytocin treatment on human prostate tissue originating from TUR-P. For information about group sizes and loss of numbers, please refer to Section 2. (a) Schematic of experimental setup: Comparison between no treatment (NT2) and cligosiban treatment (CL2). (b) Heat map representation of movements in the two analysed sections (NT2 and CL2) (white scale bar: 300 μm). (c) Left: Table showing the percentage of all movements greater than 2 standard deviations (SD > 2) within the NT2 section and the corresponding change of SD > 2 after following cligosiban treatment (CL2). Right: Graph of the cumulative frequency distribution (±SD) comparing NT2 and CL2. Cligosiban significantly decreased contractility compared to the no treatment period (n = 7) ($P < 0.01$).

Oxytocin receptor antagonists, in contrast to α_1-adrenoceptor antagonists, do not block the adrenergic pathway (thought of as the essential pathway for ejaculation): seen in our previously published work with ejaculatory tissue of the epididymis (Stadler et al., 2021). This might be the reason why the last clinical trial for premature ejaculation found that cligosiban failed to prolong intravaginal ejaculatory latency time (Althof et al., 2019). In this clinical trial, they also evaluated side effects and found them to be similar to placebo. Of course, in case of treatment with cligosiban over a prolonged period of time as it would be the case for BPH-treatment, side effects (especially any CNS effects) will have to be re-evaluated. Clinical studies involving application of oxytocin have found no, or only seldom, side effects such as dyspnea, tachycardia and nausea (Barua et al., 2017).

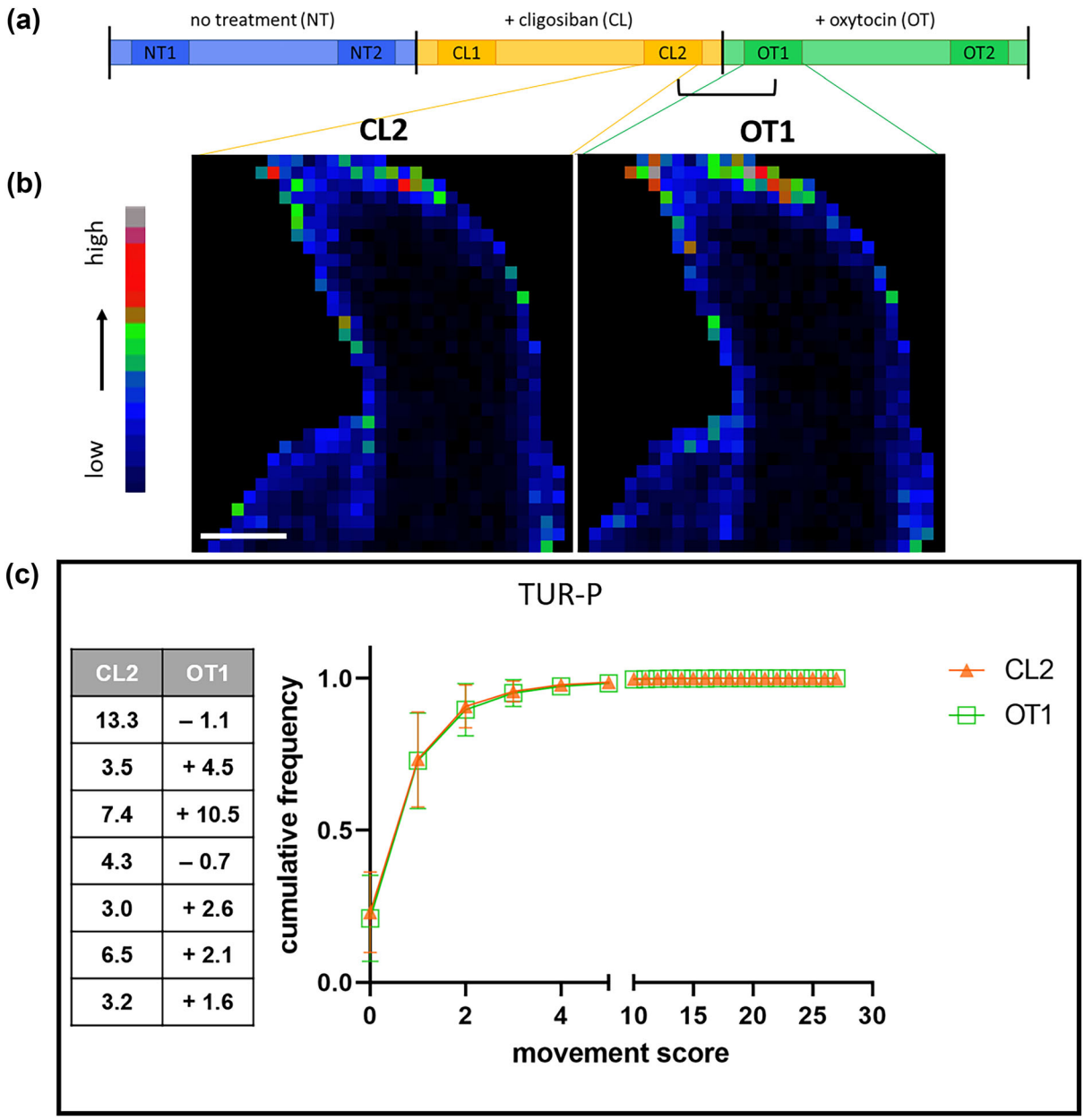


FIGURE 9 The effect of oxytocin after previous cligosiban treatment on human prostate tissue originating from TUR-P. For information about group sizes and loss of numbers, please refer to Section 2. (a) Schematic of experimental setup: comparison between cligosiban treatment (CL2) and oxytocin treatment (OT1). (b) Heat map representation of movements in the two analysed sections (CL2 and OT1) (white scale bar: 300 µm). (c) Left: Table showing the percentage of all movements greater than 2 standard deviations (SD > 2) within the CL2 section and the corresponding change of SD > 2 after following oxytocin treatment (OT1). Right: Graph of the cumulative frequency distribution (±SD) comparing CL2 and OT1. Oxytocin showed no significant effect (n = 7) ($P \geq 0.05$).

Applied dosages of the oxytocin receptor antagonist atosiban for the treatment of preterm labour result in a maximum of 600 ng·ml^{-1} in plasma (Goodwin et al., 1995) which would be equivalent to 0.6-µM atosiban. In the clinical trial for premature ejaculation applied dosages of the oxytocin antagonist cligosiban were up to 2400 mg per person, which would result in 34 mg·kg^{-1} for a 70-kg man with the plasma concentration reaching 2000 ng·ml^{-1} (Osterloh et al., 2018). The 2000-ng·ml^{-1} cligosiban would be equivalent to a concentration of 4.8-µM cligosiban.

The higher concentrations used in this study were chosen to demonstrate a maximum relaxing effect achievable with oxytocin receptor antagonists. While atosiban failed to show efficacy, the highly selective oxytocin receptor antagonist cligosiban showed considerable potential in decreasing human prostate contractility.

Our results indicate that oxytocin antagonists like cligosiban (but not atosiban) could be interesting new candidates to relax the smooth muscle cells in the diseased prostate of BPH patients, thereby alleviating lower urinary tract symptoms (LUTS).

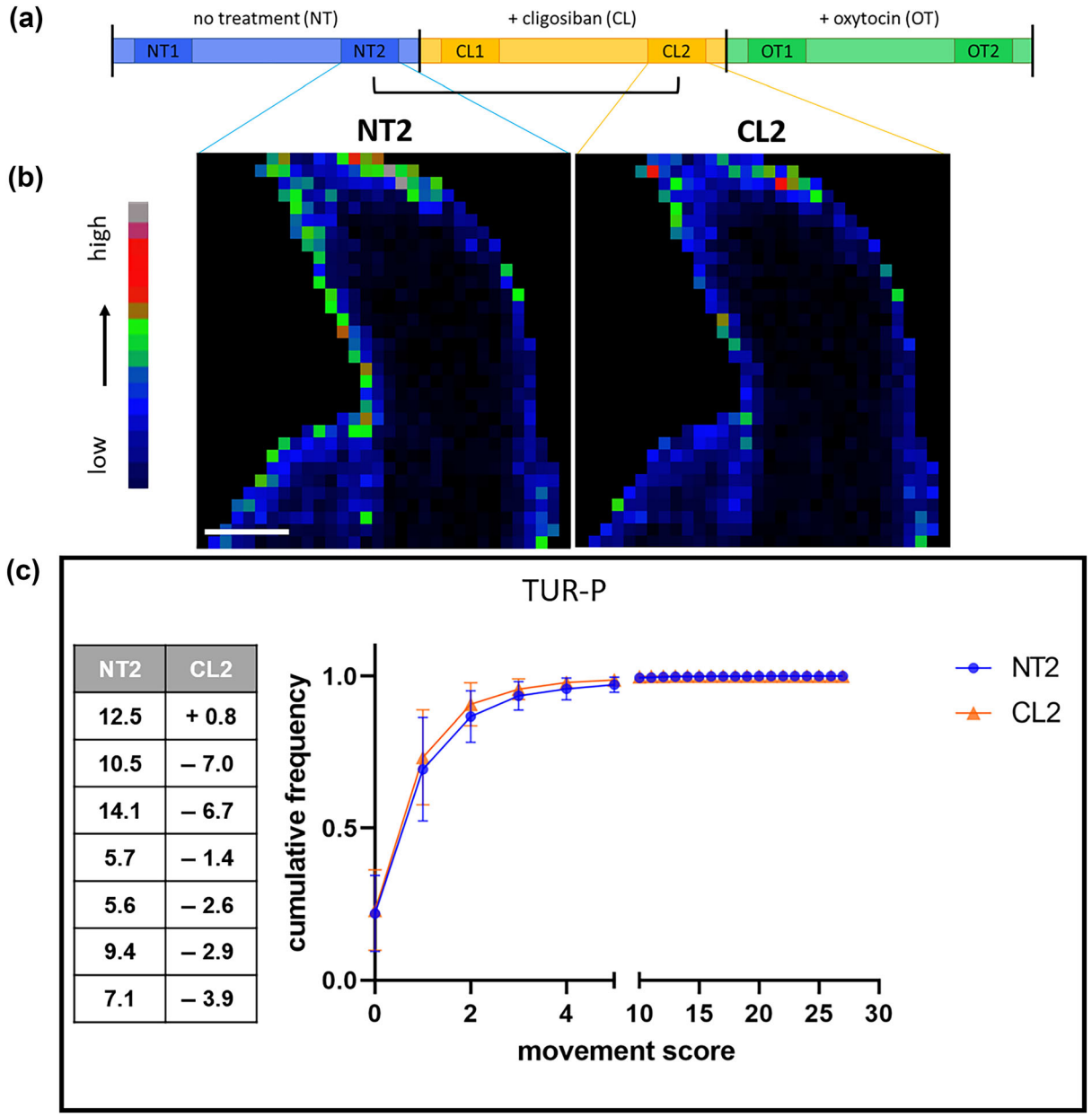


FIGURE 10 The effect of cligosiban compared to no treatment on human prostate tissue originating from TUR-P. For information about group sizes and loss of numbers, please refer to Section 2. (a) Schematic of experimental setup: Comparison between no treatment (NT2) and cligosiban treatment (CL2). (b) Heat map representation of movements in the two analysed sections (NT2 and CL2) (white scale bar: 300 μm). (c) Left: Table showing the percentage of all movements greater than 2 standard deviations (SD > 2) within the NT2 section and the corresponding change of SD > 2 after following cligosiban treatment (CL2). Right: Graph of the cumulative frequency distribution (±SD) comparing NT2 and CL2. Although tendencies for cligosiban to decrease contractility compared to the no treatment period are visible, it was not significant ($n = 7$) ($P \geq 0.05$).

AUTHOR CONTRIBUTIONS

Beatrix Bester: Conceptualization (lead); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); supervision (supporting); visualization (equal); writing—original draft (equal); writing—review and editing (equal). **Kristina Koslowa:** Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); visualization (equal); writing—original draft (equal); writing—review and editing (equal). **Ann-Catherine Gronau:** Data curation (equal); formal analysis (supporting); investigation (supporting); methodology (supporting); visualization (supporting); writing—original draft (supporting). **Andrea Mietens:** Writing—original draft (equal); writing—review and editing (equal). **Cameron J. Nowell:** Formal analysis (equal); methodology (equal); visualization (equal). **Michael Whittaker:** Supervision (supporting); writing—review and editing (equal). **Adrian Pilatz:** Resources (equal); writing—review and editing (equal). **Florian Wagenlehner:** Resources (equal); writing—review and editing (equal). **Betty Exintaris:** Conceptualization (equal); supervision (supporting); writing—review and editing (equal). **Ralf Middendorff:**

Conceptualization (lead); funding acquisition (lead); investigation (equal); resources (equal); supervision (lead); writing—original draft (equal); writing—review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

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

DATA AVAILABILITY STATEMENT

The data sets generated and/or analysed during the current study are not publicly available due to the amount and complexity of the live-imaging data but are available from the corresponding author on request.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design and Analysis, and as recommended by funding agencies, publishers and other organizations engaged with supporting research.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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