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The anxiolytic sertraline reduces the impact of psychological stress on bladder function in mice

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Abstract

Aims: To determine if treatment with the selective serotonin reuptake inhibitor (SSRI) sertraline reduces the bladder dysfunction caused by water avoidance stress in mice.

Main Methods: Adult female mice were randomly allocated to (1) Unstressed, (2) Stressed or (3) Stress + Sertraline experimental groups. Stressed mice were subjected to water avoidance for 1hr/day for 10 days and received sertraline or vehicle in drinking water, starting 10-days prior to the first stress exposure. Age matched control/unstressed mice were house under normal conditions without stress exposure. Voiding behaviour was assessed throughout the experimental protocol. After the final stress exposure, a blood sample was taken to measure plasma corticosterone levels and bladders were removed, catheterised and intravesical pressure responses recorded during distension and in response to pharmacological agents.

Key Findings: Plasma corticosterone levels in sertraline-treated animals were equivalent to unstressed controls and significantly decreased compared to the stressed group. Voiding frequency was significantly increased in the stressed group, and treatment with sertraline significantly decreased voiding frequency, however, this remained elevated compared to unstressed control animals. Bladders from stressed mice displayed enhanced maximal contractile response to the muscarinic agonist carbachol and greater release of ACh in the serosal fluid, which was reduced to control levels by sertraline treatment. Spontaneous phasic contractions were not altered by stress but were significantly reduced in bladders from sertraline treated animals, relative to controls.

Significance: These results indicate that management of voiding dysfunction caused by psychological stress may be aided by the addition of an SSRI such as sertraline.

Keywords: psychological stress; bladder; urinary frequency; water avoidance stress; sertraline

Introduction

Experimental evidence has shown that repeated psychological stress in rodents can induce bladder dysfunction, resulting in alterations in voiding which are dependent on stressor type and sex (Smith et al. , 2011, West et al. , 2020a, b). Stress induced voiding changes have been linked to both central and local mechanisms, including altered expression of corticotropin releasing factor in Barrington's nucleus, bladder wall remodelling, and altered detrusor contractility (Bilge et al. , 2008, Chang et al. , 2009, Wang et al. , 2017, West et al., 2020a, b). These experimental studies support the clinical literature which report correlations between psychological stress, including depression, anxiety and post-traumatic stress disorder, and bladder disorders such as overactive bladder and interstitial cystitis/bladder pain syndrome (Lai et al. , 2015, Lai et al. , 2016, Lee et al. , 2017).

5-Hydroxytrypamine (5-HT; serotonin) is well established as an important neurotransmitter in the CNS, and depletion this monoamine has long been theorised to play a role in depression. Experimental studies have linked depletion of 5-HT in the CNS to increases in urinary frequency and detrusor overactivity (Chiba et al. , 2016), with serotonin depletion also being postulated to play a role in the pathophysiology of OAB. In addition, 5-HT plays a role in the regulation of local bladder function, with 5-HT receptors expressed in the urothelium, detrusor muscle, as well as afferent and efferent bladder nerves. Furthermore, 5-HT receptor activation has been shown to affect detrusor and urothelial contraction, neurogenic contraction and bladder afferent firing (Chetty et al. , 2007, Konthapakdee et al. , 2019, Mittra et al. , 2007, Moro et al. , 2016).

Depression and anxiety are major contributors to the global burden of disease, the management of which often includes use of selective serotonin reuptake inhibitors (SSRI) (Lewis et al. , 2019), with sertraline being one of the most frequently prescribed (Hillhouse and Porter, 2015). While the use of SSRIs in the management of bladder dysfunction caused by psychological stress in patients has not been explored, one experimental study in rodents assessed the effects of sertraline and fluoxetine, on detrusor hypercontractility caused by forced swim test. The hypercontractility was abolished by long-term treatment with both anti-depressants (Bilge et al., 2008).

We have previously reported that repeated exposure of female mice to water avoidance stress results in a time dependent increase in urinary frequency (West et al., 2020a). This was associated with enhanced contractile bladder responses; however, it is not known if treatment

with an SSRI will be useful in managing the voiding and bladder functional changes previously reported. Therefore, the aim of the current study was to determine if treatment with sertraline can reduce the changes in voiding behaviour and local contractile mechanisms induced by repeated exposure to water avoidance stress in mice.

Materials and Methods

Animal stress model and drug treatment

All procedures were performed in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes and with the approval of the University of Queensland Animal Ethics Committee. Adult female C57Bl/6J (12-14 weeks in age; n=6 in each group) were used in this study and housed under environmentally controlled conditions, with 12-hour light-dark cycles, with access to food and water ab libitum. Mice were randomly allocated into three experimental groups: Unstressed, Stress or Stress + Sertraline.

The adult human daily sertraline dose (50mg/day) was used to calculated the equivalent dose in mice (10mg/Kg/day) based on a published dose conversion guide for humans to animals (Nair and Jacob, 2016). Sertraline was prepared as an oral suspension (National Custom Compounding, Queensland, Australia) and added to the drinking water of animals in the Stress + Sertraline group at a final concentration of 50µg/mL. Mice in the Unstressed and Stress groups received drug-free suspension (vehicle) in their drinking water. Animals received sertraline or vehicle in their drinking water for 10-days prior to and during the 10-day water avoidance stress protocol.

Water avoidance stress is a form of environmental stress that is commonly used in rodents to induce a stress response and was performed as previously described (West et al., 2020a). Briefly, mice in both stress groups were placed individually on a pedestal surrounded by water for 1 hour/day for 10 consecutive days. During this time animals were observed by the researcher and mice that jumped from the podium into the water were given the chance to climb back on the pedestal, or were placed back on the pedestal if they were unable to do so. After each 1-hour stress exposure, mice were returned to their normal housing. The unstressed group consisted of age-matched control mice housed under normal conditions and were not exposed to water avoidance stress protocols.

Open Field Test

Observation of initial animals in the sertraline treatment group suggested behaviour was altered by the drug treatment, therefore the open field test was used in all subsequent animals as a secondary outcome measure. As a result there is n=4 animals in the sertraline treated group for the open field test. Stress and stress + sertraline treated mice were placed in the open field test chamber (50 x 50 50cm) the day before drug/placebo treatment began (Day -10) for baseline measurements, the day before first stress exposure (Day 0) to determine the impact of

sertraline/placebo and on day 10 after the final stress exposure. Activity of the mice was recorded for 5 minutes and behaviour analysed in terms of number of line crossings, number of inner zone entries and number of rearings.

Voiding Pattern Analysis

Voiding behaviour was assessed as previously described to determine how stress and stress + sertraline affects urinary frequency, total voided volume, average void size and number of small voids, compared to unstressed/control animals (West et al., 2020a). VPA was performed prior to (baseline) and at intervals (1, 3, 5, 7 and 10 days) during the WAS protocol. Mice were place individually for 4 hours, at the beginning of the light cycle in normal housing cages lined with hardened ashless filter paper (Filtech; Quantitative 2um grade 225). Animals had access to food and drinking water during this time. Filter papers were collected, and urine spots detected using a Molecular Imager ChemiDoc XRS ultraviolet transilluminator (BioRad, California USA). The papers were photographed, digitized, and then analysed using Image J software. Urine spot numbers were counted as a measure of urinary frequency, in addition to measuring individual and total urine spot area, to determine average void size and total voided urine volume.

Isolated whole bladder preparation

Isolated whole bladders were used for functional bladder studies and set up as previously described (West et al. , 2018, West et al., 2020b). Animals were sacrificed by cervical dislocation 24 hours following the final water avoidance stress exposure, the bladder was isolated, and a three-way catheter was inserted through the urethra into the bladder. The urethra and ureters were ligated and the bladder was placed into a bath of gassed (95%O_2/5% CO_2) Krebs-bicarbonate solution (composition in mM: NaCl 118, $NaHCO_3$ 24.9, $CaCl_2$ 1.9, $MgSO_4$ 1.15, KCl 4.7, KH_2PO_4 1.15, and D-glucose 11.7) at 37ºC. The three-way catheter was attached to an infusion pump to allow bladder filling, a pressure transducer to record intravesical pressure, and an outflow syringe to collect intraluminal fluid and allow bladder emptying. Intravesical pressure was measured using a pressure transducer (GlobalTown Microtech, Sarasota, FL) connected to a PC via a PowerLab data acquisition system (AD Instruments, Sydney, Australia), using LabChart 7 software (AD Instruments). Following equilibration, bladder distensions were performed by intravesical infusion of saline at a rate of 30 µL/min up to a maximum pressure of 40 mmHg to assess viability, and to 20 mmHg for all further distensions.

To determine the effect of water avoidance stress and stress + sertraline on urothelial mediator release, intraluminal fluid was collected via the catheter following distension to 20 mmHg, in addition to a sample of serosal fluid. Samples were stored at -80ºC until analysis of ATP and acetylcholine (ACh) levels. Quantification of ATP and Ach was carried out using the ATP Determination Kit (Molecular Probes), and the Acetylcholine Amplex Red Assay Kit (Molecular Probes) respectively. The assays were performed according to manufacturer instructions, with luminescence and fluorescence (excitation 540, emission 590 nm) measured, using a Modulus micro-plate reader (Promega).

Following bladder distension to 20 mmHg, bladders were allowed to equilibrate/accomodate for approximately 60 minutes. Spontaneous phasic activity was measured as (1) the frequency of spontaneous contractions per minute and (2) the amplitude measured as the change in intravesical pressure from the trough to peak of the contractions during the 200s at the end of the 1 hour accommodation period, with the average amplitude recorded.

The sequence of tests performed on isolated whole bladders (EFS, pharmacological agents) was consistent between experimental groups and the same a previously described by our research group (West et al., 2018, West et al., 2020a, b). The effect of stress and stress + sertraline on nerve-evoked contractile bladder responses was assessed by electric field stimulation (EFS). The bladder was electrically stimulated (0.1ms pulse-width, 50 V) for 5 seconds, every 100 seconds at 1-20 Hz. Bladders were stimulated at each frequency until 3 consistent response were obtained and contractions were measured as the increase in intravesical pressure from baseline. EFS was repeated at 20 Hz in the absence and presence of the nitric oxide synthase inhibitor L-NNA (100 μM), atropine (1 μM) to block muscarinic receptors and αβ-methylene ATP (10 μM) to desensitize P2X receptors and thus remove cholinergic and purinergic components, respectively. Application of tetrodotoxin (0.1 μM), abolished responses to EFS, confirming the neurogenic origins of the pressure responses observed.

Intravesical pressure responses to pharmacological agents were also assessed by addition of cumulative concentrations of the muscarinic agonist carbachol, the purinergic agonists ATP (10 mM) and αβ-methylene ATP (10 μM) and relaxations to the β-adrenoceptor agonist isoprenaline following precontraction with carbachol (1 μM). Non-receptor mediated contractile bladder responses were also assessed using KCl (60 mM). All contraction and relaxation responses were measured as change in pressure from baseline. Addition of 1 μM carbachol produced a tonic contraction resulting in a steep rise in intravesical pressure,

followed by transient phasic increases in pressure. Frequency and amplitude of this phasic response was measured as described for spontaneous phasic contractions.

These experiments were performed in the same bladders, with drugs added consecutively after bladder washings, except for investigation of the relative contribution of neurotransmitters to EFS responses, in which case LNNA, atropine and αβ-methylene ATP were added consecutively without washing out and EFS response tested between each drug addition. This was performed at the end of the protocol prior to measuring non-receptor mediated contractile bladder response on addition of KCl. As recommended in previous literature when measuring bladder agonist responses, sufficient washing time was allowed to ensure return to baseline prior to further tissue stimulation (Longhurst and Uvelius, 2001).

At the time of sacrifice, a venous blood sample was taken, and plasma corticosterone levels quantified using the Corticosterone Competitive ELISA (Invitrogen) according to the manufacturer's instructions. Blood samples were collected in the morning to avoid circadian variation in corticosterone levels.

Data and statistical analysis

All experiments were randomized, with 6 mice per experimental group (except for open field test as explained above) and each experimental protocol started on a different day. Results are expressed as mean ± standard deviation (SD). Data were analysed using ordinary one-way ANOVA with Tukey multiple comparisons test or repeated measures two-way ANOVA with Bonferroni's multiple comparisons test, using GraphPad Prism version 6 software (GraphPad, San Diego, CA). Significance levels were defined as $P<0.05$ (*).

Results

Animal parameters, behaviour, and voiding

Body and bladder weight (Table 1) were not affected by water avoidance stress or stress + sertraline treatment, nor was water consumption (data not shown). The open field test was performed to assess changes in behaviours, with three parameters measured in the stress + sertraline-treated group and the stressed group. Behaviours were assessed before starting drug/placebo treatments (Day -10), before starting daily stress sessions (Day 0), and after 10-days of daily stress and treatment with sertraline/placebo (Day 10). Line crossing, inner zone entries and number of rearing episodes were not altered in the stress group (Figure 1A,C&E). Line crossing (Figure 1A) was unchanged across the treatment period and between the two groups. This indicates that the mice from both groups exhibited similar levels of activity. Mice treated with sertraline entered the inner zone more frequently than the stressed group, however, this increase in activity was not statistically significant (Figure 1 C & D). The final parameter measured was rearing behaviour which initially was similar in the two groups but was significantly reduced by sertraline both before and after inducing stress (**p = 0.003 and ***p = <0.001 respectively) (Figure 1F).

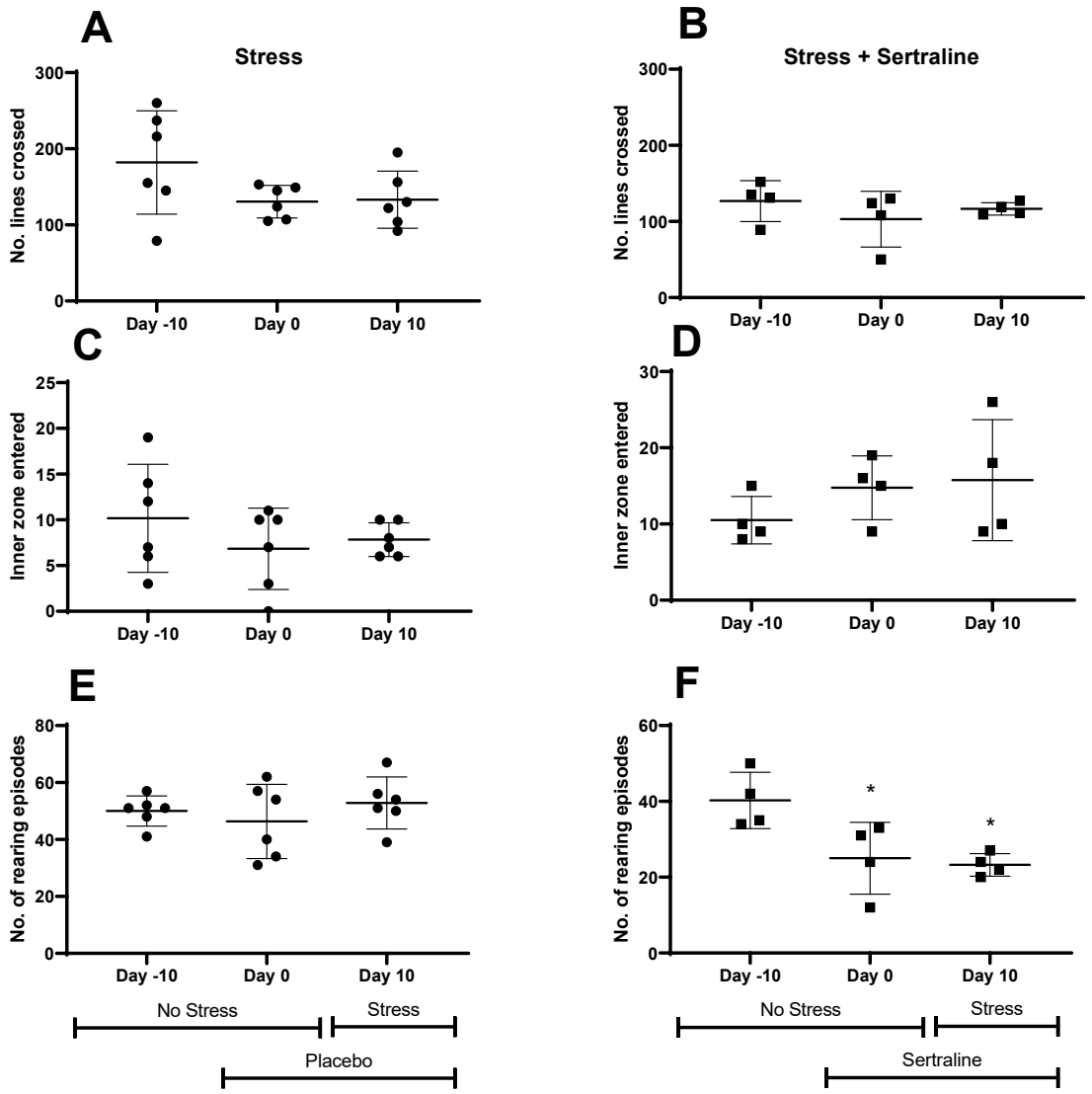

Figure 1: Open field test performed in mice from stress and stress + sertraline groups prior to drug/placebo treatment (Day -10), following 10-day drug/placebo (Day 0) and following 10-days water avoidance stress (Day 10). Data represents mean ± SD ($n \geq 4$) and was analysed using one-way ANOVA with Tukey multiple comparisons test. (*$p<0.05$ vs day-10).

Ten-day placebo/sertraline treatment prior to stress exposures did not significantly alter baseline voiding parameters, as assessed on Day 0 (Figure 2). Total voided area which represents total urine output was unchanged across the groups (Figure 2A), however urinary frequency was significantly increased in the Stressed group compared to both Unstressed and Stress + Sertraline mice (Figure 2B). However, urinary frequency was still elevated in sertraline-treated animals compared to unstressed controls. Increased urinary frequency was associated with a decrease in average void size and increase in the number of small voids

(Figure 2B&C). Plasma corticosterone levels were significantly increased in Stressed mice, however treatment with sertraline significantly reduced the increased levels of stress hormone to control levels (Figure 3A).

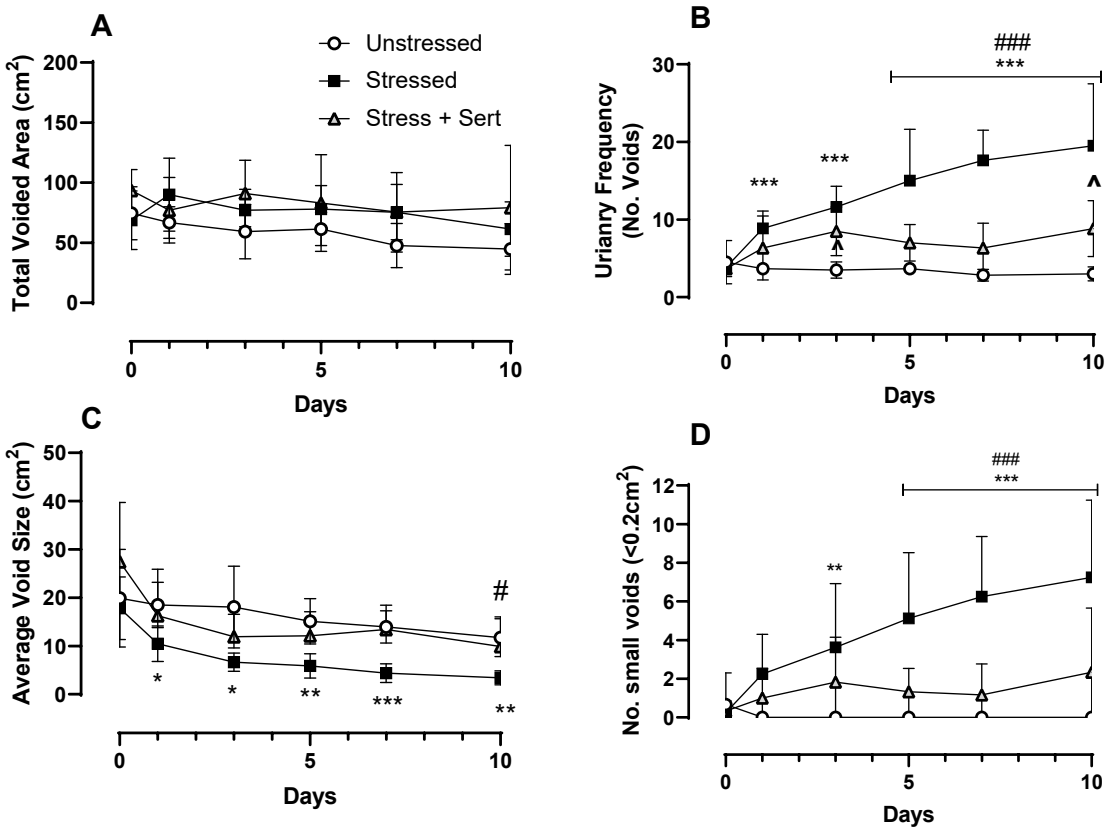

Figure 2: Voiding behaviour measured at baseline (Day 0) and during the 10-day water avoidance stress protocol. (A) Total voided area, (B) urinary frequency, (C) average void size and (D) number of small voids. Data represents mean ± SD (n=6) and was analysed using repeated measures two-way ANOVA with Bonferroni multiple comparisons test (*$p<0.05$ Stressed vs Unstressed; # $p<0.05$ Stressed vs Stress + Sertraline; ^ $p<0.05$ Unstressed vs Stress + Sertraline)

Bladder responses to agonists and electrical field stimulation (EFS)

Bladder compliance measured as the volume-pressure relationship during distention was not significantly affected by stress or sertraline (Figure 3B). Spontaneous phasic contractions were observed in all isolated bladders from unstressed and stressed mice, however spontaneous activity was only observed in 2 out of 6 bladders from the sertraline treated group. While stress alone did not significantly affect spontaneous phasic contractions measured during

accommodation, the amplitude and frequency of spontaneous contractions were significantly reduced by sertraline treatment (Figure 3C&D).

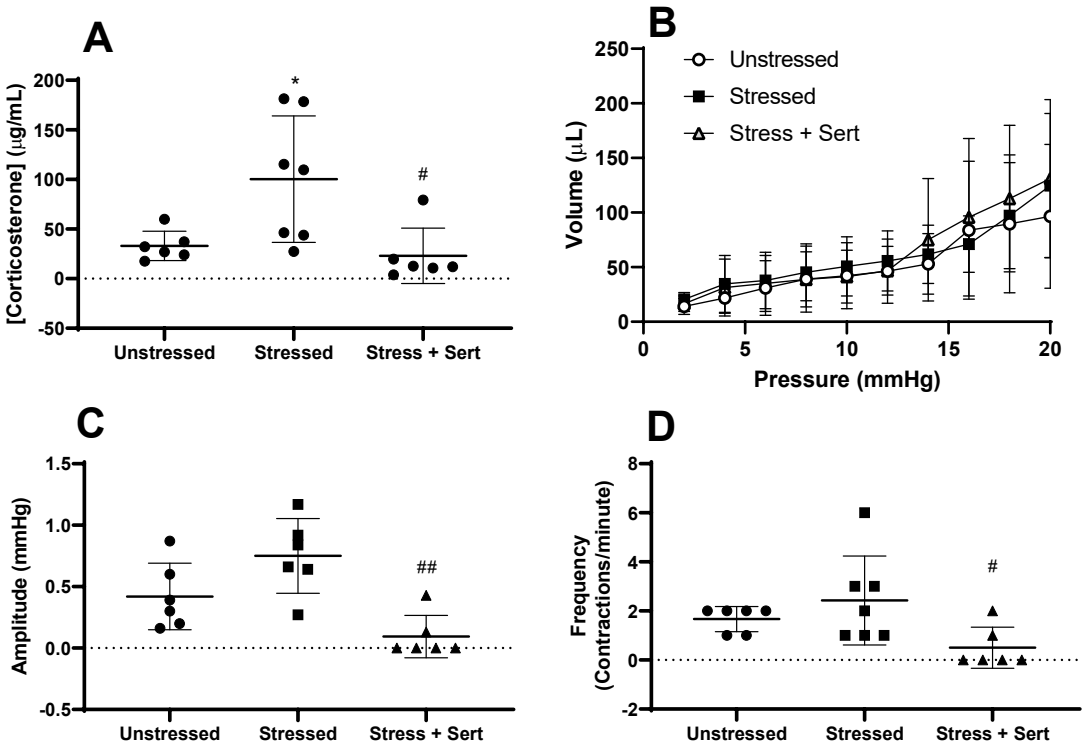

Figure 3: Effect of water avoidance stress or stress + sertraline on (A) plasma corticosterone, (B) the intravesical volume-pressure relationship as a measure of bladder compliance, and (C) the amplitude and (D) frequency of spontaneous phasic contractions measured during accommodation. Data represents mean ± SD (n=6) and was analysed using one-way ANOVA with Tukey multiple comparisons test (*p<0.05 vs unstressed; #p<0.05, ##p<0.01 vs stressed).

The muscarinic agonist carbachol produced a concentration-dependent increase in intravesical pressure in bladders from all experimental groups. However, the maximal responses were significantly increased in the Stressed and Stress + Sertraline groups compared to Unstressed animals, with no significant change in potency based on pEC_{50} values (Figure 4A and Table 1). Contractile response to KCl increased from 28.4 ± 1.91mmHg in bladders from unstressed mice to 38.2 ± 4.65mmHg with stress, however this was not statistically significant (p=0.2). The response in bladders from sertraline treated mice (30.6 ± 4.4mmHg) were similar to unstressed. When responses to carbachol were normalised to the KCl response, the maximal response was equivalent in all groups (Figure 4B). The phasic component of the carbachol (1µM) response was also quantified, and a significant increase the frequency of phasic

contractions was detected in the bladders from mice in the Stressed group compared to the Unstressed and Stress + Sertraline groups (Figure 4C). While frequency was increased in the Stress + Sertraline bladders, the magnitude was not as great as that induced by stress alone. The amplitude of phasic contractions in the presence of carbachol were unchanged (Figure 4D). Contractile response to the purinergic agonist αβmATP were similarly unchanged either by stress or stress + sertraline treatment (Unstress 17.4 ± 4.66 mmHg; Stressed 15.2 ± 3.78 mmHg; Stress + Sert 16.9 ± 6.53 mmHg).

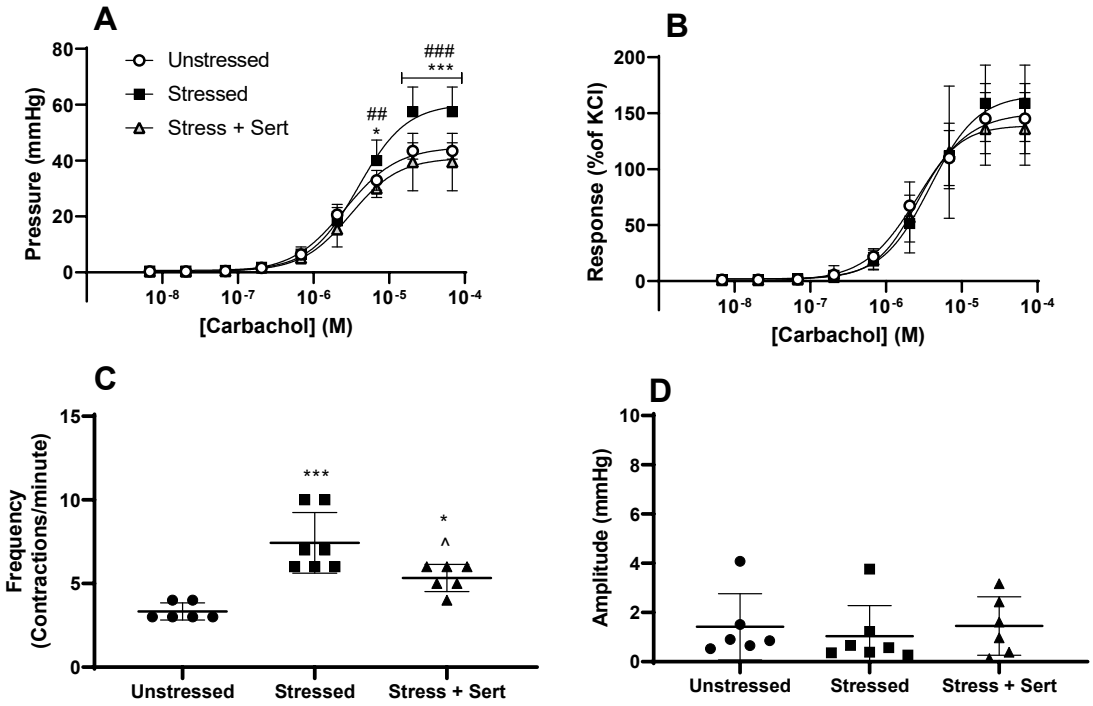

Figure 4: Effect of water avoidance stress or stress + sertraline on responses to cumulative carbachol concentrations measured as (A) change in pressure and (B) normalised to KCl response, and phasic response to 1μM carbachol measured as (C) frequency and (D) amplitude of phasic contractions. Data represents mean ± SD (n=6) and was analysed using two-way ANOVA with Bonferroni multiple comparisons test (for A and B) or one-way ANOVA with Tukey multiple comparisons test (for C and D) (A: * $p<0.05$, *** $p<0.001$ Stressed vs Unstressed; ## $p<0.01$, ### $p<0.001$ Stressed vs Stress + Sertraline. C: * $p<0.05$, *** $p<0.001$vs Unstressed; ^ $p<0.05$ vs Stressed).

Nerve mediated contractile bladder responses were investigated using EFS, which resulted in comparable frequency-dependent increases in intravesical pressure in bladders from each of the groups (Figure 5A). However, when the pressure response to EFS was normalised to the KCl response, a significant reduction in nerve evoked contractions in the Stressed group was

detected, this was statistically significant at 10 and 20 Hz stimulation (Figure 5B). Nitric oxide has been shown to act as an inhibitory neurotransmitter in the bladder, however addition of the nitric oxide synthase inhibitor L-NNA (100μM) did not affect responses to EFS in any of the experimental groups. However, addition of the muscarinic agonist atropine (1μM) reduced responses by 11.4 ± 2.0% and desensitization of purinergic receptors with αβmATP (10μM) reduced responses by a further 50.9 ± 2.59% in unstressed controls. The relative contributions of muscarinic and purinergic components to nerve evoked contractions was not affected by either stress or stress + sertraline treatment (data not shown).

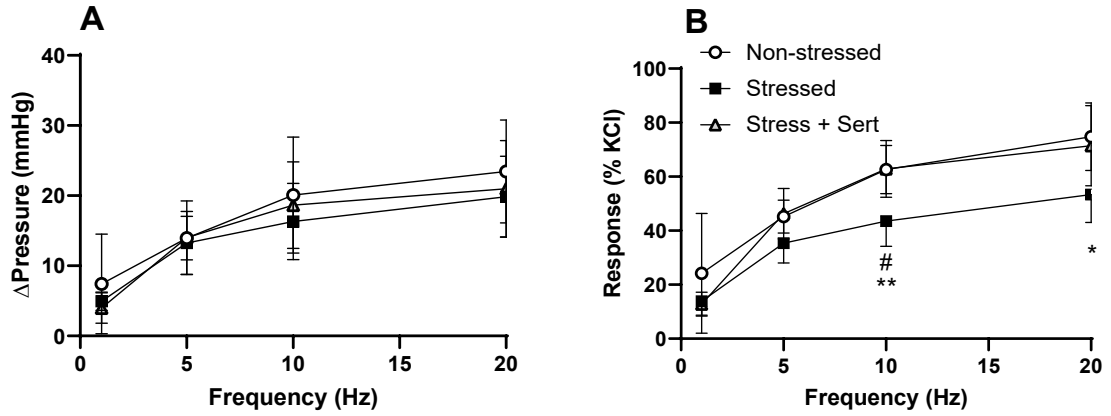

Figure 5: Responses to electric field stimulation in isolated whole bladders from control, stressed and stress + sertraline mice. (A) Pressure responses and (B) responses normalised to KCl response. Data is presented as mean ± SD (n=6) and was analysed using two-way ANOVA with Bonferroni multiple comparisons test (*p<0.05, **p<0.01 Stressed vs Control; #p<0.05 Stressed vs Stress + Sertraline).

Bladder relaxation in response to the β-adrenoceptor agonist isoprenaline following precontraction with carbachol (1μM) was also assessed. There was a significant reduction in the maximal relaxation to isoprenaline in Stress and Stress + Sertraline groups compared to Unstressed controls when presented as the pressure change (Figure 6A), however this decrease was not statistically significant when expressed as a percentage of the pre-contraction (Figure 6B, Table 1). The potency of isoprenaline was similar in all groups (Table 1).

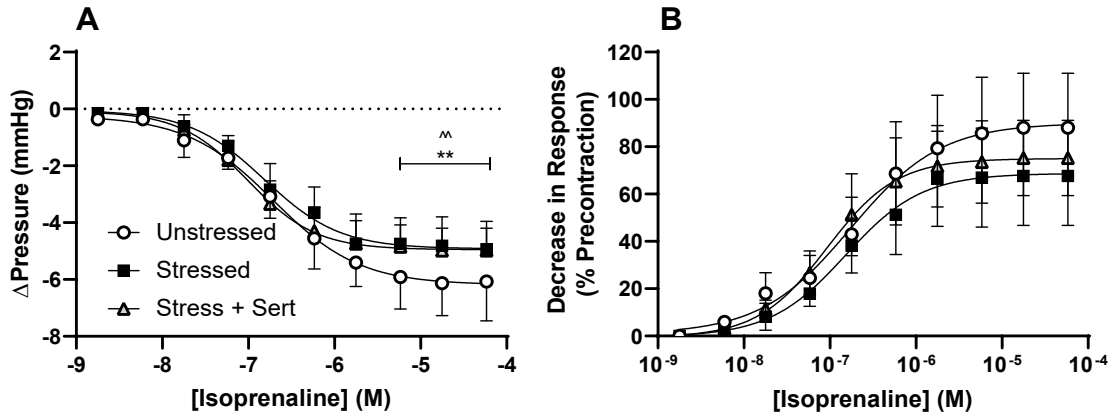

Figure 6: Effect of water avoidance stress or stress + sertraline on responses to isoprenaline following pre-contraction with 1μM carbachol. Data presented as (A) change in pressure (mmHg) and (B) change in pressure as a % of the precontraction to carbachol. Data is expressed as mean ± SD (n=6) and was analysed using two-way ANOVA with Bonferroni multiple comparisons test (**p<0.01 Unstressed vs Stressed; ^^p<0.01 Unstressed vs Stress + Sertraline).

Urothelial ATP & ACh release during bladder filling

The release of urothelial mediators was assessed in both the luminal and serosal fluid when the bladders were filled to 20mmHg. The levels of ATP and Ach were 50-fold and 15-fold greater respectively in the serosal fluid compared to the luminal levels. While neither the stress exposure nor sertraline treatment altered serosal ATP or intraluminal ATP or ACh (Figure 7A-C), release of ACh into the serosal fluid was significantly enhanced following stress compared to the unstressed and sertraline treatment group (Figure 7D).

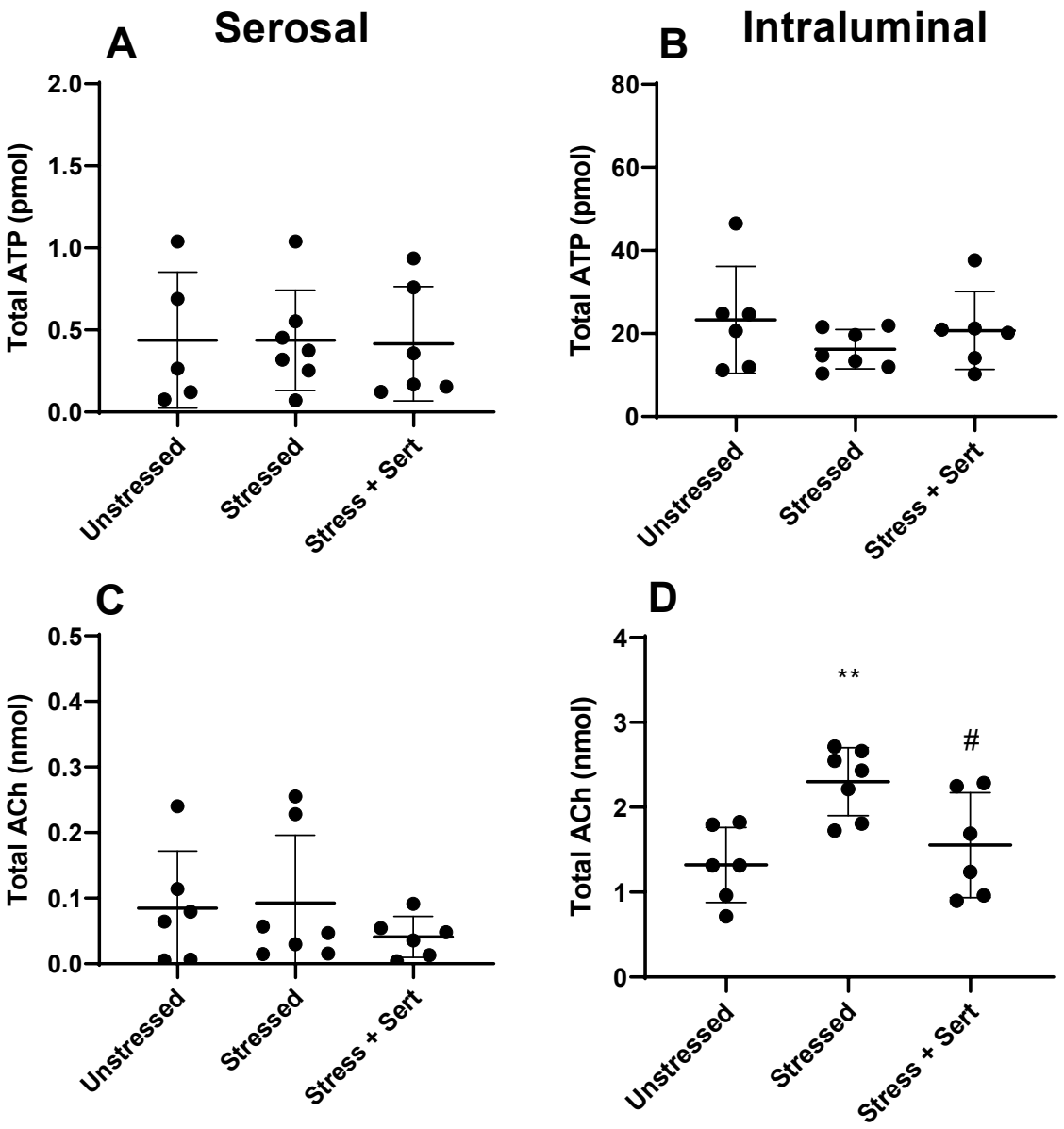

Figure 7: Effect of water avoidance stress or stress + sertraline on total release of (A-B) ATP and (C-D) ACh into the intraluminal and serosal fluid following distension of isolated whole bladders to 20mmHg. Data is presented as mean ± SD (n=6) and was analysed using one-way ANOVA with Tukey-Kramer multiple comparisons test (** $p<0.01$ Stressed vs Unstressed; #$p<0.05$ Stress + Sertraline vs Stressed)

Discussion

We have previously reported that water avoidance stress produces an overactive bladder phenotype, with increased voiding frequency evident and changes in bladder physiology, including an increase in detrusor muscle contractility (West et al., 2020a). These voiding changes are like those reported clinically, with increased urinary frequency common among individuals experiencing chronic stress (Lai et al., 2015, Lai et al., 2016, Zhang et al. , 2013). SSRIs such as sertraline are commonly used in the clinical management of anxiety and depression. They have a low side-effect profile compared to other anti-depressants, however their benefit in management of stress induced voiding dysfunction is unknown. Experimental studies have linked depletion of serotonin in the CNS to increases in urinary frequency and detrusor overactivity (Chiba et al., 2016), with serotonin depletion being postulated to play a role in the pathophysiology of OAB. This would suggest that an SSRI may be of therapeutic benefit in managing the voiding dysfunction associated with stress.

The primary outcome of this study was to determine if treatment with sertraline would limit the impact of water avoidance stress on voiding and we observed that sertraline treatment significantly decreased urinary frequency over the course of 10 days of stress exposure compared to the untreated water avoidance stress group. While this change alludes to effective management of voiding dysfunction caused by stress, it must be noted that urinary frequency remained significantly elevated in the sertraline treatment group compared to the unstressed controls. There have been no experimental or clinical studies which have investigated the effects of sertraline on urinary frequency with stress, however from what we know of the actions of SSRIs like sertraline and the role of serotonergic mechanisms in regulating lower urinary tract function, the benefits of sertraline observed in the current study are not surprising.

The open field test was used to measure the anxious and depressive state of mice following water avoidance stress and sertraline treatment. Interestingly, the WAS model was not associated with anxiety like behaviour, with similar behaviours observed at baseline and following repeated stress. This supports a study by Hassan et al (2014), who similarly found 7-days WAS in mice had little influence on behaviour. Line crossing and therefore ambulatory distance was unchanged between the groups in the current study which means that the mice are not affected by inactivity or pain (Gould, 2009). Inner zone entering was increased in the sertraline treated mice, although not significantly. This parameter measures the natural aversion rodents have to exposed areas, and because of the increased activity in the ‘inner zone’ by the

sertraline treated mice, it can be concluded that these mice were suffering less from anxiety-like behaviour (Carola et al. , 2002). Rearing was significantly reduced in the sertraline treated group compared to the stressed group, over the course of the stress period. There is still some debate as what type of behaviour rearing indicates, however, the general consensus is that rearing is indicative of anxiety (Borta and Schwarting, 2005), therefore, sertraline treatment reduced anxiety like behaviour which has been reported previously in rodents (Bikomo et al. , 2017).

Sertraline, as an SSRI increases the amount of available serotonin (Anderson et al. , 2005), an important neurotransmitter which plays a role in the control of the lower urinary tract (Andersson and Pehrson, 2003). Serotonergic neurons are present in several regions of the CNS which are involved in providing descending inhibition to the bladder, including the Onuf nuclei and the lumbosacral autonomic nuclei which innervate the urethral sphincters (Andersson and Pehrson, 2003). The raphe nuclei of the medulla has been shown to contain serotonergic neurons with descending projections to the bladder, urethra and external urethral sphincter (Espey et al. , 1998).

Serotonin when administered experimentally, either results in inhibitory or facilitatory affects, depending on which receptor dominates. An experimental study on cats found that serotonin induces external urethral sphincter activity to prevent leakage of urine during the storage phase (Espey et al., 1998). This is also supported by other animal studies which have observed Onuf nucleus stimulation with serotonin to cause contraction of the external urethral sphincter (Thor, 2003). Paraneurone-neural signalling within the urethra uses 5-HT to simulate sensory pathways to the CNS (Kullmann et al. , 2018), with efficient voiding dependent on sensory information from the urethra (Danziger and Grill, 2016, Shafik et al. , 2003). It could therefore be postulated that 5-HT depletion in the urethra may contribute to the voiding symptoms observed with stress, therefore sertraline therefore via its direct action as an SSRI may restore 5-HT signalling in the urethra and aid in improving voiding behaviours. Simulation of 5-HT receptors suppress the processing of afferent input from the bladder and results in excitation of sympathetic and inhibition of parasympathetic preganglionic neurons (De Groat and Ryall, 1967, Espey et al. , 1992, Ryall and DeGroat, 1972).

Clinical studies have observed urinary hesitancy and retention after beginning treatment with sertraline (Lowenstein et al. , 2007). This may be due to the number of serotonergic neurons present within the neural centres of micturition. The increased control of the external urethral

sphincter by the serotonergic neurons, may explain why the mice treated with sertraline showed decreased voiding frequency compared to the stressed group. Both clinical and experimental studies have shown that duloxetine, a noradrenaline-serotonin reuptake inhibitor, is of benefit in the treatment of OAB (Steers et al. , 2007, Wrobel et al. , 2020).

As reported previously, repeated WAS exposure increased maximum contractile responses to carbachol, an effect that appears to result from a general increase in contractility as this change was not evident when responses were normalised to the KCl contraction. We found that sertraline treatment abolished this change in detrusor contractility, an effect that has also been reported in rodents with detrusor hypercontractility induced by the forced swim test (FST). The study found that sertraline significantly decreased contractility to carbachol, potassium and EFS (Bilge et al., 2008). Due to the inhibition of KCl-stimulated contractile response by sertraline, the study suggested that the mode of action of the SSRI was not on the receptors themselves, but by the inhibition of downstream signalling or contractile mechanisms (Bilge et al., 2008). The inhibition of contractile responses after sertraline treatment has been studied in a number of different isolated tissues. A study using rat aortic rings found that calcium channels are inhibited by sertraline, causing relaxation of aortic smooth muscle, an effect that was independent of serotonergic mechanisms (Becker et al. , 2004). Similarly, a recent study using isolated rat hearts to investigate ischemia and reperfusion reported that sertraline had a strong vasodilatory effect on coronary vessels (Grotthus and Szelag, 2019). Contractile responses to noradrenaline, KCl and EFS in rat isolated vas deferens was also inhibited by sertraline treatment similarly via effects on calcium channels (Kalyoncu et al. , 1999). Addition of a voltage-gated calcium channel activator restored the contractile responses when sertraline was washed out of the isolated tissue bath. The actions of sertraline on calcium channels have been proposed as a mechanism additional to its action as an SSRI. Therefore, the inhibitory action of sertraline on calcium channels may have contributed to the overall decrease in contractility witnessed in the sertraline treatment group. A more recent study treated female rats with corticosterone as a model of stress. The authors reported that this caused detrusor inflammation and overactivity as well as depression. They found that treatment with the serotonin-noradrenaline reuptake inhibitor, duloxetine, improved detrusor overactivity and depression via central mechanisms (Wrobel et al., 2020).

Despite the increase in the contractile responses to carbachol and potassium, the raw pressure responses to EFS were unchanged by stress, as seen previously in the WAS model (West et al., 2020a) and were similarly unchanged by sertraline treatment. However, when EFS responses

were normalised to the KCl contraction, nerve evoked responses were significantly depressed in the stress group but not in the sertraline treated group. This suggests that repeated WAS exposure alters efferent innervation and/or neurotransmission, an effect that was overcome by sertraline treatment. Previous research has shown that efferent neurotransmission in the bladder is regulated by serotonergic mechanisms, with pre-junctional 5-HT receptors reported to potentiate neurotransmission in several species (Barras et al. , 1996, Bhattacharya et al. , 2004, Chapple et al. , 2004, Sellers et al. , 2000); and may account for the differences observed here with sertraline treatment.

Evidence suggests that sympathetic stimulation of beta-adrenoceptors in the detrusor muscle may be important to the storage phase by inhibiting bladder contraction (Andersson and Arner, 2004). In the current study there was a tendency for reduction in the relaxation response to the beta-adrenoceptor agonist isoprenaline in bladders from stressed mice compared to those from unstressed animals, although this was not statistically significant when expressed as a percentage of the carbachol precontraction. However, this may affect normal storage function in stressed mice and contribute to increased urinary frequency observed in this group.

It is well established that detrusor smooth muscle exhibits spontaneous phasic activity (Levin et al. , 1986) which is shown to be myogenic in origin (Buckner et al. , 2002). Spontaneous activity in isolated detrusor muscle has been reported to be dependent on both calcium entry mechanisms and potassium channels and spontaneous contractions are modulated by the presence of the urothelium (Buckner et al., 2002, Vahabi et al. , 2013). Sertraline treatment had a significant effect on spontaneous phasic activity and phasic responses to the muscarinic agonist carbachol. After treatment with sertraline, frequency of phasic activity was significantly decreased compared to the stressed group. While the anti-depressant effects of sertraline are typically attributed to inhibition of serotonin reuptake mechanisms, the biological actions of SSRIs are more complex with evidence of additional inhibition of sodium, potassium and calcium channels (Deak et al. , 2000, Frizzo, 2017, Lee et al. , 2012, Ohno et al. , 2007). While it has previously been reported that sertraline has effects on general contractility, as discussed above, no studies have measured spontaneous activity after treatment with this drug. It may be postulated that due to the reported actions of sertraline on calcium and potassium channels, and the role of these channels in regulating the frequency and amplitude of phasic activity in the bladder; this may explain the decreased spontaneous activity observed in the sertraline treated group (Becker et al., 2004, Bilge et al., 2008, Ohno et al., 2007). The

therapeutic benefit of sertraline in improving voiding behaviour with stress exposure may in part be due to a reduction in phasic activity in the bladder.

During the storage phase, stretch of the urothelium prompts release of signalling mediators including ATP and ACh, which play an important role in regulation of normal bladder function, and changes in their release has been linked to bladder pathologies (Birder et al. , 2010). While ATP levels were unchanged in the present study, release of ACh into the serosal fluid was significantly enhanced following stress exposure, while sertraline treatment prevented this change. Research has shown that bladder afferent neurons express M_2, M_3 and M_4 muscarinic receptor subtypes, suggesting a role for urothelial ACh in regulating sensory processing in the bladder (Nandigama et al. , 2010). M_2 receptors are reportedly involved in bladder afferent activation in rats with spinal cord injury (Matsumoto et al. , 2012), while the therapeutic benefit of the anti-cholinergic darifenacin has been linked to its ability to desensitize bladder afferents (Iijima et al. , 2007). Greater release of ACh during bladder filling in stressed animals could therefore stimulate bladder sensory nerves, triggering micturition at lower filling volumes. The ability of sertraline to reduce urothelial ACh release could therefore contribute to its ability to improve voiding behaviour.

Conclusions

Repeated exposure to water avoidance stress induced bladder overactivity. Treatment with the anxiolytic sertraline decreased the effects of stress on voiding behaviour, although not to unstressed control levels. However, sertraline treatment abolished stress-induced changes in bladder contractile responses and release of urothelial ACh. These results indicate that management of bladder dysfunction caused by psychological stress may be aided by the addition of an SSRI such as sertraline.

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Table 1: Baseline animal parameters and whole bladder responses to carbachol and isoprenaline in unstressed, stressed and stress + sertraline mice. Data is presented as mean ± SD for weight and maximal response and mean with 95% confidence limits for pEC_{50} values (n=7) analysed using one-way ANOVA with Tukey multiple comparisons test. (*** $p<0.001$ vs unstressed, ^^^ $p>0.001$ vs stress).

	Unstressed	**Stress**	**Stress + Sertraline**
	Animal Parameters		
Body Weight (g)	20.8±0.31	21.8±0.96	20.9±0.58
Bladder Weight (mg)	15.4±2.57	17.8±3.61	16.3±2.80
	Isolated Whole Bladder Responses		
Carbachol			
pEC_{50}	5.59 (5.52-5.66)	5.41 (5.32-5.51)	5.51 (5.36-5.67)
Maximal response (mmHg)	44.9±2.52	60.3±5.34***	40.9±5.46 ^^^
Isoprenaline			
pEC_{50}	6.76 (6.2-7.09)	6.83 (6.52-7.09)	7.06 (6.79-7.28)
Maximal response (% decrease)	90.01±14.09	68.3±8.75	75.2±7.50

Figure Legends

Figure 1: Open field test performed in mice from stress and stress + sertraline groups prior to drug/placebo treatment (Day -10), following 10-day drug/placebo (Day 0) and following 10-days water avoidance stress (Day 10). Data represents mean ± SD ($n \geq 4$) and was analysed using one-way ANOVA with Tukey multiple comparisons test. (*$p<0.05$ vs day-10).

Figure 2: Voiding behaviour measured at baseline (Day 0) and during the 10-day water avoidance stress protocol. (A) Total voided area, (B) urinary frequency, (C) average void size and (D) number of small voids. Data represents mean ± SD (n=6) and was analysed using repeated measures two-way ANOVA with Bonferroni multiple comparisons test (*$p<0.05$ Stressed vs Unstressed; # $p<0.05$ Stressed vs Stress + Sertraline; ^ $p<0.05$ Unstressed vs Stress + Sertraline)

Figure 3: Effect of water avoidance stress or stress + sertraline on (A) plasma corticosterone, (B) the intravesical volume-pressure relationship as a measure of bladder compliance, and (C) the amplitude and (D) frequency of spontaneous phasic contractions measured during accommodation. Data represents mean ± SD (n=6) and was analysed using one-way ANOVA with Tukey multiple comparisons test (*$p<0.05$ vs unstressed; #$p<0.05$, ##$p<0.01$ vs stressed).

Figure 4: Effect of water avoidance stress or stress + sertraline on responses to cumulative carbachol concentrations measured as (A) change in pressure and (B) normalised to KCl response, and phasic response to 1μM carbachol measured as (C) frequency and (D) amplitude of phasic contractions. Data represents mean ± SD (n=6) and was analysed using two-way ANOVA with Bonferroni multiple comparisons test (for A and B) or one-way ANOVA with Tukey multiple comparisons test (for C and D) (A: * $p<0.05$, *** $p<0.001$ Stressed vs Unstressed; ## $p<0.01$, ### $p<0.001$ Stressed vs Stress + Sertraline. C: * $p<0.05$, *** $p<0.001$vs Unstressed; ^ $p<0.05$ vs Stressed).

Figure 5: Responses to electric field stimulation in isolated whole bladders from control, stressed and stress + sertraline mice. (A) Pressure responses and (B) responses normalised to KCl response. Data is presented as mean ± SD (n=6) and was analysed using two-way ANOVA with Bonferroni multiple comparisons test (*$p<0.05$, **$p<0.01$ Stressed vs Control; #$p<0.05$ Stressed vs Stress + Sertraline).

Figure 6: Effect of water avoidance stress or stress + sertraline on responses to isoprenaline following pre-contraction with 1μM carbachol. Data presented as (A) change in pressure (mmHg) and (B) change in pressure as a % of the precontraction to carbachol. Data is expressed as mean ± SD (n=6) and was analysed using two-way ANOVA with Bonferroni multiple comparisons test (**$p<0.01$ Unstressed vs Stressed; ^^$p<0.01$ Unstressed vs Stress + Sertraline).

Figure 7: Effect of water avoidance stress or stress + sertraline on total release of (A-B) ATP and (C-D) ACh into the intraluminal and serosal fluid following distension of isolated whole bladders to 20mmHg. Data is presented as mean ± SD (n=6) and was analysed using one-way ANOVA with Tukey-Kramer multiple comparisons test (** $p<0.01$ Stressed vs Unstressed; #$p<0.05$ Stress + Sertraline vs Stressed)