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The effect of oxytocin and an enriched environment on anxiety-like behaviour and corticosterone levels in a prenatally stressed febrile seizure rat model

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ABSTRACT

Background: Febrile seizures (FS) are a neurological abnormality which occur following a fever that has resulted from a systemic infection and are characterised by convulsions. These convulsions occur due to abnormally increased signalling of interleukin-1 beta, resulting in increased neuronal hyper-excitability. Furthermore, exposure to prenatal stress has been shown to exacerbate seizure duration, elicit anxiety-like behaviour and corticosterone levels. Oxytocin is a neuropeptide with anxiolytic, social bonding, and stress regulation effects. Therefore, the aim of the study was to assess whether oxytocin can attenuate the anxiety-like behaviour and increased corticosterone in rat offspring exposed to prenatal stress and FS.

Method: Sprague Dawley rats were mated. On GND14, prenatal stress was induced on pregnant dams for 1 hr/7 days. On PND 14, rat pups were injected with lipopolysaccharide (LPS, 200 μg/kg, i.p.) followed 2.5 h later by an i.p. injection of kainic acid (KA, 1.75 mg/kg). Oxytocin (1 mg/kg) was induced via different routes (intraperitoneal or intranasal) as well an enriched environment between PND 22–26. The enriched environment included larger cages (1560 cm^2) with only 4 pups per cage, compared to those groups not receiving enrichment (646 cm^2), as well as cardboard rolls and plastic toys. On PND 27–33 the light/dark box and elevated plus maze were used to assess anxiety-like behaviour. On PND 34 all rats were euthanized using a sharp guillotine, trunk blood and hypothalamic tissue were collected for neurochemical analysis (ELISA kit).

Results: Our findings confirmed that exposure to both prenatal stress and febrile seizures resulted anxiety-like behaviour and significantly higher plasma corticosterone concentrations compared to their counterparts. Environmental enrichment was significantly effective in attenuating the increased basal corticosterone levels and anxiety-like behaviour seen in the prenatally stressed FS rat. Although direct administration of oxytocin showed higher significance in reducing corticosterone plasma levels when compared to the enriched environment. Furthermore, hypothalamic oxytocin levels were not significant in rat exposed to environmental enrichment while oxytocin treatment showed a significant effect when compared to their counterparts.

Conclusion: Therefore, oxytocin administration during early postnatal development shows great potential in reversing the effects of prenatal stress and its subsequent exacerbation of FS.

1. Introduction

Febrile seizures (FS) are a neurological abnormality that occurs following a fever when the core body temperature rises severely due to an underlying infection and can be characterised by neuronal hyper-excitability (Gatti et al., 2002; Dubé et al., 2009; Riazi et al., 2010; Qulu et al., 2012). These seizures occur commonly in three-month to five-year-old children (Freeman, 1980; Waruiru and Appleton, 2004), with a prevalence of 2–5% (Waruiru and Appleton, 2004; Heida et al., 2009; Hwang et al., 2015; Kariuki et al., 2017). Complex and status epilepticus seizures have been shown to lead to cognitive and behavioural deficits later in life, as well as increased susceptibility to epilepsy (Heida et al., 2009; Qulu et al., 2012). Following infection and fever, abnormally increased signalling of the pro- inflammatory cytokine,

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interleukin-1 beta (IL-1β), leads to the development of FS by causing increased neuronal firing and neuronal hyper-excitability, thereby progressively increasing excitatory neurotransmission and, in addition, also decreases inhibitory neurotransmission, thus mediating the pathogenesis of febrile convulsions (Dubé et al., 2005, Mazarati, 2005, Heida et al., 2009, Wendorff and Zeman, 2011).

Previous studies have shown that FS are exacerbated in rat offspring exposed to prenatal stress (Qulu et al., 2012; Qulu et al., 2015). The hypothalamic- pituitary- adrenal (HPA) axis is the main hormone system involved in the response to stress and plays a role in maintaining physiological homeostasis (Smith and Vale, 2006; Stephens and Wand, 2012). The HPA axis releases glucocorticoids, cortisol in humans or corticosterone in rats, into the blood in response to stress (Smith and Vale, 2006; Stephens and Wand, 2012; Guest et al., 2013). These glucocorticoids play a role in the body's "fight or flight" response, after which the increase in glucocorticoid levels is counteracted by negative feedback mechanisms involving binding to glucocorticoid and mineralocorticoid receptors in the pituitary and the brain (Smith and Vale, 2006; Stephens and Wand, 2012). This negative feedback mechanism ensures that glucocorticoid levels are kept within a narrow range, the disruption of which can lead to an increased risk of pathologies (Smith and Vale, 2006; Stephens and Wand, 2012).

Despite maternal glucocorticoid levels being naturally elevated during pregnancy, prenatal stress causes a further increase in glucocorticoid levels, thus leaving the foetus more prone to excessive cortisol exposure (Charil et al., 2010; Davis et al., 2011; Harris and Seckl, 2011; Guest et al., 2013). Exposure to increased glucocorticoid levels reduces the density of mineralocorticoid and glucocorticoid receptors in the foetal brain (Buitelaar et al., 2003; Charil et al., 2010; Davis et al., 2011; Qulu et al., 2015); therefore, exposure to excessive levels of glucocorticoids also saturates these receptors, resulting in excess circulating glucocorticoids (Harris and Seckl, 2011, de Kloet et al., 2014, Cassim et al., 2015). These increased basal glucocorticoids could subsequently potentiate neuroinflammation in the central nervous system and promote the release of cytokines, which in turn, increases susceptibility to FS (Sorrells et al., 2009; Frank et al., 2013; Frank et al., 2015). A prenatally stressed FS rat model has been shown to exhibit an exaggerated seizure response, the highest levels of corticosterone, and anxiety-like behaviour compared to non-stressed controls (Qulu et al., 2012; Qulu et al., 2015).

The neuropeptide, oxytocin (OXT), has anti-inflammatory, anxiolytic, as well as anti-stress effects, in that it is able to attenuate HPA axis activity and decrease corticosterone levels following prenatal stress (Heinrichs et al., 2003; Olausson et al., 2003; Uvnas-Moberg and Petersson, 2005; Lee et al., 2007, Cardoso et al., 2013, Rault et al., 2013). The anxiolytic effects of OXT in rodents have been determined in several behavioural tests for anxiety such as the four-plate (Ring et al., 2006); the light dark box (Blume et al., 2008); the elevated zero maze (Ring et al., 2006); and the elevated plus maze (Windle et al., 1997; Blume et al., 2008) where most animals showed signs of reduced anxiety-like behaviour. Several studies have also reported reduced plasma corticosterone concentrations following OXT administration (Windle et al., 1997; Petersson et al., 1999; Olausson et al., 2003; Windle et al., 2004).

An enriched environment, which has also previously been shown to ameliorate the effects of prenatal stress on the HPA axis, as well as anxiety- like behaviour, is defined as a combination of "complex inanimate objects and social stimulation" (Van Praag et al., 2000; Morley-Fletcher et al., 2003; Mccreary, Metz, 2016). It is essentially understood as allowing the subject to be content in choosing their own behaviour to promote their psychological well-being (Mccreary, Metz, 2016; Vega-Rivera et al., 2016). Studies have shown that exposure of prenatally stressed rats to an enriched environment results in the upregulation of glucocorticoid receptors and reverses the prolonged secretion of corticosterone in response to stress (Morley-Fletcher et al., 2003; Emack and Matthews, 2011; Li et al., 2012; Mccreary, Metz, 2016).

Since previous studies have shown that offspring exposed to prenatal stress have fewer OXT producing neurons in the hypothalamus than those who were not exposed (de Souza et al., 2013, Wang et al., 2018), and taken together the effects of both OXT and an enriched environment on the prenatal stress-induced malfunctioning HPA axis and anxiety-like behaviour, it would be interesting to examine whether the administration of OXT or exposure to an enriched environment has an effect on a prenatally stressed FS rat model.

Since OXT exerts different effects on the peripheral and central oxytocinergic systems, and the peripheral and central systems are separated by the blood brain barrier, there is a possibility that the site of action could be different if OXT were administered either systemically or centrally (Talegaonkar and Mishra, 2004; Ring et al., 2006). Therefore, the aim of this study was to administer systemic (intraperitoneal) or central (intranasal) OXT to determine if either mode of OXT administration is capable of attenuating the anxiety-like behaviour and increased corticosterone levels caused by prenatal stress and FS; or to expose the prenatally stressed FS rat model to an enriched environment to determine whether environmental enrichment (EE) can reverse the anxiety-like behaviour and increased corticosterone levels caused by prenatal stress and FS.

2. Experimental procedures

2.1. Animals

The experimental protocols and procedures conducted in this study were approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (Ethics ref. no: AREC/034/018 M).

A total of 120 Sprague Dawley rats were used in this study as follows: 16 female and 8 male rats were obtained from the Biomedical Resource Unit (BRU) at the University of KwaZulu- Natal. These rats were used to breed 96 male pups that were used for the study. All the extra pups were kept undisturbed with their dam until they were weaned on postnatal day (PND) 14, after which they were safely returned to the BRU. Food and water were readily available, and the animals were housed under standard laboratory conditions: 22 ± 1 °C room temperature, 70% humidity, and a 12-hour light/dark cycle (lights on at 06:00, off at 18:00).

2.2. Mating

The female rats' oestrus cycles were assessed prior to mating. The oestrus cycle of a normal female rat spans 4–5 days (Nelson et al., 1982; Hubscher et al., 2005), thus vaginal smears were done daily for 4–5 days to track the oestrus cycle (Cassim et al., 2015; Qulu et al., 2015). Female rats were housed together in a colony cage for about a week to allow for the synchronisation of their oestrus cycles (Cassim et al., 2015). When the females were in pro-oestrus, two females were placed together in large, opaque cages (646 cm2), followed by the introduction of a male rat, to allow for mating to occur. A vaginal plug, or the presence of sperm in a vaginal smear the following morning indicated successful mating and was regarded as gestational day (GND) 0. The male rat was removed from the cage following successful mating (Qulu et al., 2012; Cassim et al., 2015; Qulu et al., 2015).

2.3. Prenatal stress protocol

On GND 14, the pregnant females were divided into two groups namely non-stressed (NS) and stressed (S). The NS pregnant rats remained undisturbed in their cages and received food and water ad libitum throughout the pregnancy, while the S pregnant females were taken daily to a separate room and following acclimatisation to the new environment for 1 h, were placed in rodent restrainers for 1 h daily, for a total of 7 days (GND 14 –20). These S pregnant rats were then returned to their home cages at the end of the stress period each day (Qulu et al.,

2012; Cassim et al., 2015; Qulu et al., 2015).

2.4. Febrile seizure induction

Following the birth of the rat pups, the pups remained undisturbed with their dams until postnatal day (PND) 14, after which 96 pups were divided into the following groups (n = 24): non-stressed -saline (NS); non-stressed - febrile seizure (NS-FS); stressed - saline (S); and stressed - febrile seizure (S-FS). The FS groups were placed in a new cage and taken to the experimental room. Following acclimatisation to the new environment for 1 h, the induction of seizures began. This involved an intraperitoneal injection of 50 μg/kg lipopolysaccharide (LPS) (Sigma-Aldrich, St Louis, MI, USA), after which pups were returned to their dams to allow for suckling and grooming. After 2.5 h, the pups were removed from their dams again and injected intraperitoneally with 0.44 mg/kg kainic acid (KA) (Sigma-Aldrich, St Louis, MI, USA). The control groups received an equal volume of saline (Qulu et al., 2012; Cassim et al., 2015; Qulu et al., 2015). The pups were weaned on PND 21.

2.4.1. Assessment of febrile seizures

Following the KA injection, convulsive behaviour was monitored for 1 h, and seizure severity was scored according to Table 1 (Heida et al., 2004; Ojewole, 2008; Qulu et al., 2015).

2.5. Treatments

For each treatment, the FS, and saline groups (from the respective non-stressed and stressed groups) were further divided into control (which received no treatment) (C); enriched environment exposure (EE); intraperitoneal OXT (IP); and intranasal OXT (IN) groups.

2.5.1. OXT administration

Systemic administration: The groups receiving intraperitoneal OXT were placed in a new cage and taken to the experimental room. Following acclimatisation to the new environment for 1 h, the administration began. A dose of 1 mg/kg of OXT (Sigma-Aldrich, USA) was dissolved in 0.9% saline and administered as an intraperitoneal injection for 5 consecutive days, from PND 22 to PND 26. A 29 gauge 0.5 mm insulin needle was used for all injections (Olausson et al., 2003).

Central administration: The groups receiving intranasal OXT were placed in a new cage and taken to the experimental room. Following acclimatisation to the new environment for 1 h, the administration began. A dose of 1 mg/kg of OXT was dissolved in 0.9% saline and administered intranasally for 5 consecutive days, from PND 22 to PND 26. This involved the inhalation of droplets of drug (OXT) intranasally via a micropipette (Wang et al., 1998). This was not an invasive procedure hence did not require anaesthesia. Half of the volume of OXT was administered into each nostril. The micropipette was loaded with the OXT, and the tip of the micropipette was placed near the rat's nostril at a 45° angle, while the rat was held inverted with its ventral side facing upward and its neck parallel to the floor. The OXT was then slowly ejected from the micropipette, to form a small droplet at the end of the pipette tip. The droplet was placed close enough to the rat's nostril so that the OXT could be inhaled. The rat was held in this inverted position and until the droplet was inhaled. The inhalation of the droplet took ~ 2–3 s as the droplet was extremely small when ejected from the micropipette. Following the inhalation of this droplet, about 2–3 s later, more of the OXT in the pipette tip was ejected to form another small droplet to be inhaled by the rat, through the other nostril. Any snorted-out droplets were replaced. The rat was held in the aforementioned position while the ejection of these droplets of OXT from the pipette tip was repeated and alternated between nostrils until the full dose and volume of OXT was administered. Any snorted-out droplets were replaced.

Table 1
Scoring of seizure severity.

Stage	Response
0	No response
2	Ear and facial twitching
1	Loss of postural control
3	Myoclonic jerks and rearing
4	Clonic convulsions – animal falling on its side
5	Repeated severe tonic-clonic convulsions

2.5.2. Enriched environment exposure

The pups receiving the enrichment were placed in environmentally enriched cages on PND 21 when the pups were weaned. The enriched environment included larger cages (1560 cm2) with only 4 pups per cage, compared to those groups not receiving enrichment (646 cm2), as well as cardboard rolls and plastic toys. This allowed for more space for play and explorative behaviour. Shredded paper was also added to the bedding. All novel objects in the cage were cleaned with the rest of the cage and replaced if necessary. The rats could freely roam and play in these cages until the behavioural tests were conducted.

2.6. Behavioural tests

2.6.1. Light/dark box

The rats were subjected to behavioural training between PND 27 and 29; and the test commenced on PND 31. The light/dark box is a behavioural apparatus which assesses exploratory or anxiety-like behaviour in rodents by exposing them to brightly illuminated light compartments and one dark compartment (Bourin and Hascoet, 2003). Studies have shown that when rats are exposed to a novel environment, they display either exploratory or anxiety- like behaviour (Spencer et al., 2005). Exploratory behaviour is viewed as the rat being able to navigate a novel environment and explore the different compartments of the apparatus as well as a greater amount of time taken to find the dark box (Qulu et al., 2015). Anxiety-like behaviour is viewed as an inability/disinterest in performing these tasks i.e., the rat finds the darker compartment much more quickly (less exploration of the different compartments) and remains in the dark compartment for a longer time (Qulu et al., 2015). The light/dark box that was used was made of plexi glass (100 ×30×30 cm3) with illuminating light (Qulu et al., 2015). This apparatus was divided into four compartments of equal size with the first three compartments brightly lit with an illuminating bulb and the fourth compartment opaque with no light bulb (Qulu et al., 2015). During the training sessions between PND 27 and 29, the compartments were not partitioned, but partitions were inserted during the test phase on PND 31, where the rats were tested on their ability to navigate and explore the different compartments. The light dark box was wiped and cleaned with 70% ethanol after each rat, to prevent olfactory cues from affecting the behaviour of the subsequently tested rat.

2.6.1.1. Training sessions (PND 27–29). The animals were taken to the experimental room 1 h before the start of the training session to allow for acclimatisation to the new environment (Qulu et al., 2015). The training procedure involved each rat being placed at the far corner of the first brightly lit compartment facing away from the rest of the compartments to be explored (Qulu et al., 2015). The rat was allowed 60 s to find the dark compartment (Qulu et al., 2015). If the rat failed to do so in the stipulated time, it was physically taken and placed in the dark compartment for 60 s before being returned to the home cage, and training resumed the next day (Qulu et al., 2015).

2.6.1.2. Test (PND 31). The animals were taken to the experimental room 1 h before the start of the training session to allow for acclimatisation to the new environment (Qulu et al., 2015). Transparent plexi glass with small openings at the bottom was used to partition the light/dark compartments (Qulu et al., 2015). Scoring was as follows: It was

rated as anxiety-like behaviour, if the rat spent the entire 60 s in the first compartment in an immobile state or if the rat spent most of the time in the dark compartment, instead of exploring the light compartments. It was rated as explorative behaviour, if the rat explored all the compartments, spending a greater percentage of the time exploring the light compartments. Exploratory behaviour was quantified as the percentage of total time spent exploring the light compartments. Following completion of the test, the rats were returned to their home cages.

2.6.2. Elevated plus maze test

On PND 33, anxiety-like behaviour was also tested using the elevated plus maze test. This apparatus was raised 50 cm above the floor and consisted of an open platform in the centre of a 4-arm maze in which 2 open arms were opposed perpendicularly by 2 enclosed arms (40 ×10 cm). Confinement to the closed arms provided security and represented anxiety-like behaviour, while exploration in the open arms represented explorative behaviour (File, 1993). The rats were placed in the middle of the platform and were allowed 5 min to explore the maze. Scoring was as follows: It was rated as anxiety-like behaviour, if the rat spent most of the time in the closed arms, instead of exploring the open arms. It was rated as explorative behaviour, if the rat explored all the arms, spending a greater percentage of the time exploring the open arms. Exploratory behaviour was quantified as the percentage of total time spent exploring the open arms. Following completion of the test, the rats were returned to their home cages.

2.7. Euthanasia

On PND day 34, the rats were taken to the autopsy room 1 h before decapitation to allow for acclimatisation. The rats were decapitated using a sharp guillotine; trunk blood was collected for the corticosterone enzyme-linked immunosorbent assay (ELISA) (Qulu et al., 2012; Qulu et al., 2015). Hypothalamic tissue was removed, weighed, and snap frozen in liquid nitrogen, followed by storage in a bio-freezer at −80 °C (Qulu et al., 2015) for neurochemical analysis (OXT ELISA).

2.8. Enzyme-Linked Immunosorbent Assay (ELISA)

2.8.1. Plasma corticosterone analysis

Plasma concentrations of corticosterone were measured from trunk blood (from all groups) using a commercially available rat corticosterone (CORT) ELISA kit (Elabscience Biotechnology Inc., Wuhan, China), according to the manufacturer's instructions. Blood was collected in heparin-coated test tubes and was centrifuged at 4 °C for 15 min at 10,000 rpm. The supernatant (plasma) was collected for the assay. Briefly, 50 μL of standard working solution (of concentrations 200, 100, 50, 25, 12.5, 6.25, 3.13, and 0 ng/mL) and samples were added to two wells each (to run in duplicate), on a 96 well micro-ELISA plate pre-coated with rat CORT, followed by the immediate addition of 50 μL of biotinylated detection Ab (specific for rat corticosterone) working solution to each well. The plate was then incubated at 37 °C for 45 min, to allow for rat corticosterone in the standard/sample to compete with a fixed amount of rat CORT on the solid phase support, for sites on the biotinylated detection Ab specific to rat CORT, after which the liquid in the wells was removed, followed by three wash steps to remove excess conjugate and unbound standard/sample. The wash steps were carried out using a plate washer (BioTek, Highland Park, USA). This involved the removal of the solution in each well, after which 350 μL of wash buffer was added to each well. This was allowed to soak for 1–2 min, after which the solution in each well was removed and the plate was patted dry against an absorbent paper. This was followed by the addition of 100 μL of horseradish peroxidase (HRP) conjugate working solution to each well, and a 30-minute incubation at 37 °C. This was to allow for the enzyme to attach to the unbound rat CORT on the plate. The solution in each well was then removed, followed by five wash steps. Thereafter, 90 μL of substrate reagent was added to each well, followed by a 15-minute incubation at 37 °C. This allowed for an enzyme-substrate reaction to occur, causing the solution in these wells to turn blue in colour. This reaction was then terminated upon the addition of 50 μL stop solution, causing the solution in these wells to turn yellow in colour. A micro-plate reader (set to 450 nm) was then used to determine the optical density (OD) of each well. The OD value was inversely proportional to the concentration of rat corticosterone. The concentration of rat corticosterone in samples was calculated by comparing the OD of the samples with the standard curve.

2.8.2. Hypothalamic oxytocin analysis

Hypothalamic tissue was prepared for analysis by thoroughly removing excess blood, by rinsing the tissue in ice-cold phosphate buffered saline (0.01 M, pH = 7.4). The tissue samples were then weighed and homogenised in phosphate buffered saline (the volume of which depended on the tissue weight) on ice, using an ultrasonic sonicator (Qsonica, MODEL CML-4), so as to disrupt the cell wall and release the contents of the cell. The homogenates were then centrifuged (Hermle LASEC, Germany) at 4 °C for 5 min at 50,000 x g to obtain the supernatant. The supernatant was transferred to new Eppendorf tubes, to be used for the assay. Hypothalamic OXT concentrations (from all groups) were measured using a commercially available OXT (OT) ELISA kit (Elabscience Biotechnology Inc., Wuhan, China), according to the manufacturer's instructions. Briefly, 50 μL of standard working solution (of concentrations 1000, 500, 250, 125, 62.5, 31.25, 15.63, and 0 pg/mL) and samples were added to two wells each (to run in duplicate), on a 96 well micro-ELISA plate pre-coated with OT, followed by the immediate addition of 50 μL of biotinylated detection Ab (specific for OXT) working solution to each well. The plate was then incubated at 37 °C for 45 min, to allow for OXT in the standard/sample to compete with a fixed amount of OXT on the solid phase support, for sites on the biotinylated detection Ab specific to OXT, after which the liquid in the wells was removed, followed by three wash steps to remove excess conjugate and unbound standard/sample. The wash steps were carried out using a plate washer (BioTek, Highland Park, USA). This involved the removal of the solution in each well, after which 350 μL of wash buffer was added to each well. This was allowed to soak for 1–2 min, after which the solution in each well was removed and the plate was patted dry against an absorbent paper. This was followed by the addition of 100 μL of HRP conjugate working solution to each well, and a 30-minute incubation at 37 °C. This was to allow for the enzyme to attach to the unbound OT on the plate. The solution in each well was then removed, followed by five wash steps. Thereafter, 90 μL of substrate reagent was added to each well, followed by a 15-minute incubation at 37 °C. This allowed for an enzyme-substrate reaction to occur, causing the solution in these wells to turn blue in colour. This reaction was then terminated upon the addition of 50 μL stop solution, causing the solution in these wells to turn yellow in colour. A micro-plate reader (set to 450 nm) was then used to determine the OD of each well. The OD value was inversely proportional to the concentration of OXT. The concentration of OXT in samples was calculated by comparing the OD of the samples with the standard curve.

2.9. Statistical analysis

The data were analysed using GraphPad Prism software (version 7). Normality and Gaussian Distribution was determined using the Smirnov-Kolmogorov test. Non-parametric data (seizure severity) were analysed using a Mann Whitney test. Three-way factorial analysis of variance (ANOVA) was performed on the behavioural, plasma corticosterone, and hypothalamic OXT data with stress, FS, and treatment as components of variation. Significant main effects and interactions were followed by Tukey's multiple comparisons post hoc test. Differences were considered significant when p value < 0.05.

3. Results

3.1. Seizure severity

Seizure severity was assessed in rats with induced febrile seizures. The following groups were assessed: non-stressed (NS-FS) and stressed (S-FS). Seizure intensity was scored from 0 – no effect, to 5 – repeated severe tonic-clonic convulsions as per Table 1 above. There was a stress effect as the S-FS rats reached stage 5 tonic-clonic convulsions while the NS-FS rats only progressed to stage 3 myoclonic jerks (NS-FS vs. S-FS, $p < 0.0001$). Data are presented as mean ± SEM (n = 24 rats per group) in Fig. 1.

3.2. Behavioural tests

3.2.1. Light dark box

Anxiety-like behaviour was assessed using the light dark box, which was expressed as a measure of exploration, quantified as the percentage of total time spent exploring the light compartments. The following groups were assessed: NS-C; NS-EE; NS-IP; NS-IN; NS-FS-C; NS-FS-EE; NS-FS-IP; NS-FS-IN; S-C; S-EE; S-IP; S-IN; S-FS-C; S-FS-EE; S-FS-IP; and SFS- IN (refer to experimental procedures section above for full group names). A 3-way ANOVA showed that there was a stress effect ($F(1,3) = 13.83$, $p = 0.0004$) since the stressed control groups spent significantly less time exploring the light compartments compared to the non-stressed control groups. There was an overall febrile seizure effect as the FS animals spent less time exploring the light compartments compared to the saline groups ($F(1,3) = 14.5$, $p = 0.0003$). There was also an overall significant treatment effect which was able to reverse the stress effects resulting in more time spent exploring the light compartments compared to their controls ($F(3, 3) = 440.7$, $p < 0.0001$). There was also an interaction between stress and treatment ($F(3, 3) = 17.78$, $p < 0.0001$). Data are presented as mean ± SEM (n = 6 rats per group) in Fig. 2.

3.2.2. Elevated plus maze test

Anxiety-like behaviour was assessed in the elevated plus maze, which was expressed as a measure of exploration, quantified as the percentage of total time spent exploring the open arms. The following groups were assessed: NS-C; NS-EE; NS-IP; NS-IN; NS-FS-C; NS-FSEE; NS-FS-IP; NS-FS-IN; S-C; S-EE; S-IP; S-IN; S-FS-C; S-FS-EE; S-FS-IP; and S-FS-IN. A 3-way ANOVA showed that there was a stress effect ($F(1,3) = 30.69$, $p < 0.0001$). The stressed control groups spent significantly less time exploring the open arms compared to the nonstressed control groups. There was a FS effect since the FS groups spent significantly less time exploring the open arms compared to the saline groups ($F(1, 3) = 12.37$, $p = 0.0007$). There was also an overall OXT treatment effect which was able to reverse the stress effects resulting in more time spent exploring the open arms compared to the control groups, in both non-stressed animals and stressed animals ($F(3,3) = 33.6$, $p < 0.0001$). Data are presented as mean ± SEM (n = 6 rats per group) in Fig. 3.

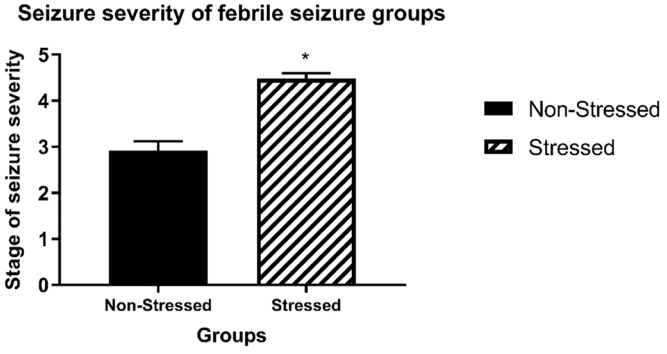

Fig. 1. : Mean seizure severity in non-stressed (NS) and stressed (S) febrile seizure (FS) groups. There was a significant difference in the seizure severity between prenatally stressed and non-stressed rats * (NS-FS vs. S-FS, $p < 0.0001$). Data are presented as mean ± SEM (n = 24 rats per group).

3.3. ELISA

3.3.1. Plasma corticosterone analysis

Corticosterone concentrations were assessed in the plasma of the rats. The following groups were assessed: NS-C; NS-EE; NS-IP; NS-IN; NS-FS-C; NS-FS-EE; NS-FS-IP; NS-FS-IN; SC; S-EE; S-IP; S-IN; S-FS-C; S-FS-EE; S-FS-IP; and S-FS-IN. A 3-way ANOVA showed that there was a stress effect ($F(1,3) = 110.8$, $p < 0.0001$). The stressed control groups had significantly higher plasma corticosterone concentrations compared to non-stressed control groups. There was a FS effect since the FS groups had significantly higher plasma corticosterone concentrations compared to the saline groups ($F(1, 3) = 12.66$, $p = 0.0012$). There was an overall significant treatment effect in that treatment was able to reverse the effects of stress resulting in lower plasma corticosterone concentrations compared to the control groups, in both nonstressed and stressed animals ($F(3,3) = 110.3$, $p < 0.0001$). There were also interactions between stress and treatment ($F(3, 3) = 30.5$, $p < 0.0001$); stress and FS ($F(1, 3) = 12.66$, $p = 0.0012$); and stress, FS and treatment ($F(3, 3) = 9.254$, $p = 0.0001$). Data are presented as mean ± SEM (n = 3 rats per group) in Fig. 4.

3.3.2. Hypothalamic oxytocin analysis

Oxytocin concentrations were assessed in the hypothalamic tissue of the rats. The following groups were assessed: NS-C; NS-EE; NS-IP; NS-IN; NS-FS-C; NS-FS-EE; NS-FS-IP; NS-FS-IN; S-C; S-EE; S-IP; S-IN; S-FS-C; S-FS-EE; S-FS-IP; and S-FS-IN. A 3-way ANOVA showed that although there was no significant effect of the EE, there was an overall significant OXT treatment effect which resulted in significantly higher hypothalamic OXT concentrations compared to the controls, in both non-stressed and stressed animals ($F(3, 3) = 53.13$, $p < 0.0001$). Data are presented as mean ± SEM (n = 3 rats per group) in Fig. 5.

4. Discussion

In this study we investigated whether the administration of OXT (intraperitoneal or intranasal) or exposure to an enriched environment has an effect on a prenatally stressed FS rat model.

We exposed the pregnant Sprague Dawley females to an hour of restraint stress from GND14 for 1 week. After the pups' birth, FS were induced on PND 14, and treatment (OXT or EE) was administered from PND 22 – 26. Thereafter, the rats' anxiety-like behaviour was assessed.

Our results show that although both OXT administration and exposure to an EE were successful in attenuating the increased basal corticosterone levels and anxiety-like behaviour seen in the prenatally stressed FS rat model, the effects of OXT administration were more robust.

Our findings also show that exposure to prenatal stress resulted in significantly more severe seizures as well as anxiety-like behaviour in both the light dark box and elevated plus maze. This result is in agreement with previous studies by Qulu *et.al.* (2012; 2015) who also reported an exaggerated seizure response and anxiety-like behaviour in offspring that were exposed to prenatal stress (Qulu et al., 2012; Qulu et al., 2015). This is due to the increased basal corticosterone levels previously reported in prenatally stressed offspring (Qulu et al., 2015), which is also confirmed with our chemical analysis of plasma corticosterone concentrations in prenatally stressed rats. This increased basal corticosterone concentration in prenatally stressed offspring is due to increased exposure of the foetus to maternal glucocorticoids because of the reduced activity and expression of 11β -HSD2, which ultimately results in a dysregulated HPA axis in the offspring causing higher basal glucocorticoid secretion in the offspring (Charil et al., 2010; Davis et al.,

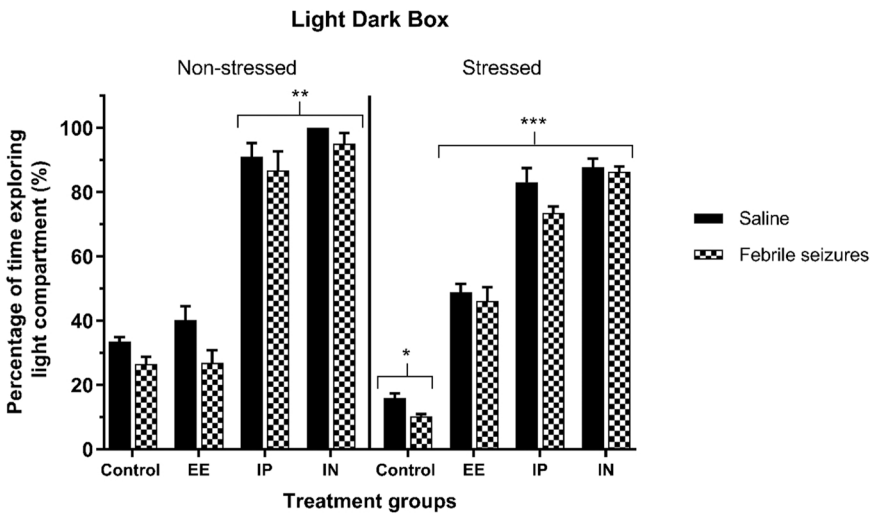

Fig. 2. : Graph showing exploratory behaviour in the light dark box, which was quantified as the percentage of total time spent exploring the light compartments. Eight subgroups were used for the non-stressed study while another 8 subgroups for the stressed study. Significant differences were observed between * (NS-C vs. S-C, $p = 0.0190$); * (NS-FS-C vs. S-FS-C, $p = 0.0483$); * *(NSC vs. NS-IP, $p < 0.0001$); * *(NS-FS-C vs. NS-FS-IP, $p < 0.0001$); ** (NS-C vs. NS-IN, $p < 0.0001$); * *(NS-FS-C vs. NS-FS-IN, $p < 0.0001$) and * ** (S-C vs. S-EE, $p < 0.0001$); * ** (SFS- C vs. S-FS-EE, $p < 0.0001$); ***(S-C vs. S-IP, $p < 0.0001$); ***(S-FS-C vs. S-FS-IP, $p < 0.0001$); ***(S-C vs. S-IN, $p < 0.0001$); ***(S-FS-C vs. S-FS-IN, $p < 0.0001$). Data are presented as mean ± SEM (n = 6 rats per group).

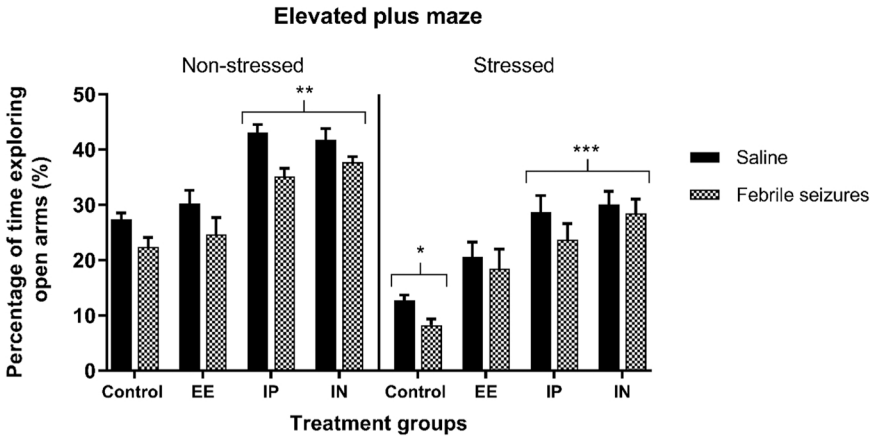

Fig. 3. : Graph showing exploratory behaviour in the elevated plus maze, which was quantified as the percentage of total time spent exploring the open arms. Eight subgroups were used for the non-stressed study while another 8 subgroups for the stressed study. Significant differences were observed between * (NS-C vs. S-C, $p = 0.0014$); * (NS-FS-C vs. S-FS-C, $p = 0.0025$); * *(NSC vs. NS-IP, $p = 0.0004$); * *(NS-FS-C vs. NS-FS-IP, $p = 0.0113$); * * (NS-C vs. NS-IN, $p = 0.0017$); * *(NS-FS-C vs. NS-FS-IN, $p = 0.0007$) and * ** (S-C vs. S-IP, $p = 0.0003$); * ** (SFS- C vs. S-FS-IP, $p = 0.0006$); * ** (S-C vs. S-IN, $p < 0.0001$); * ** (S-FS-C vs. S-FS-IN, $p < 0.0001$). Data are presented as mean ± SEM (n = 6 rats per group).

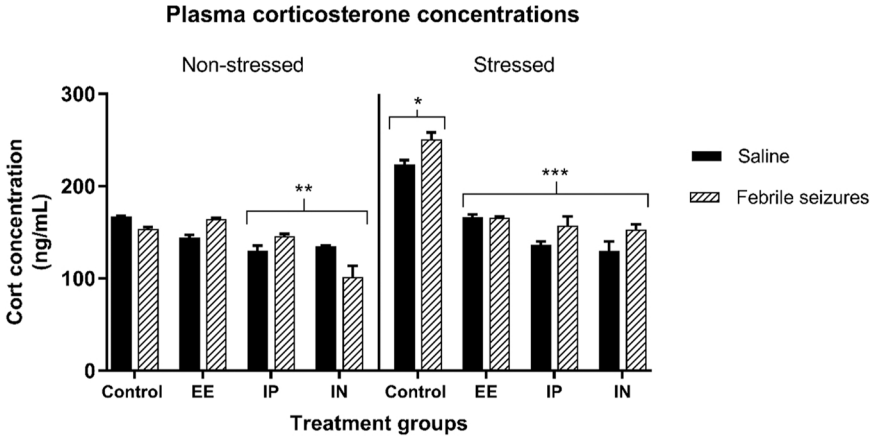

Fig. 4. : Graph showing plasma corticosterone concentrations (ng/mL). Eight subgroups were used for the non-stressed study while another 8 subgroups for the stressed study. Significant differences were observed between * (NS-C vs. S-C, $p < 0.0001$); * (NS-FS-C vs. S-FS-C, $p < 0.0001$); * *(NSC vs. NS-IP, $p = 0.0056$); * *(NS-C vs. NS-IN, $p = 0.0244$); * *(NS-FS-C vs. NS-FS-IN, $p < 0.0001$) and * ** (S-C vs. S-EE, $p < 0.0001$); * ** (S-FS-C vs. S-FS-EE, $p < 0.0001$); * ** (S-C vs. S-IP, $p < 0.0001$); * ** (S-FS-C vs. S-FS-IP, $p < 0.0001$); * ** (S-C vs. S-IN, $p < 0.0001$); * ** (S-FS-C vs. S-FS-IN, $p < 0.0001$). Data are presented as mean ± SEM (n = 3 rats per group).

2011; Harris and Seckl, 2011; Guest et al., 2013). The increase in basal glucocorticoids could subsequently potentiate neuroinflammation in the central nervous system and promote the release of cytokines (Sorrells et al., 2009; Frank et al., 2013; Frank et al., 2015). This occurs because innate immune cells in the brain, called microglia, enter a state characterised as primed, following exposure to glucocorticoids (Frank et al., 2015). These primed microglia release exaggerated levels of inflammatory cytokines (such as IL-1β) when stimulated by LPS, hence could have the same response when stimulated by infection (Frank et al., 2015). This is also consistent with findings by Qulu *et. al.* (2012) where it was shown that rat pups exposed to prenatal stress and FS showed increased plasma IL-1β levels (Qulu et al., 2012). The increased plasma IL-1β levels could subsequently lead to the exaggerated seizure response seen in the prenatally stressed rats due to increased binding to IL-1RI, thus activating the Src family of kinases, which in turn phosphorylate the NR2A/B subunit of NMDA receptors (Viviani et al., 2003, Dubé

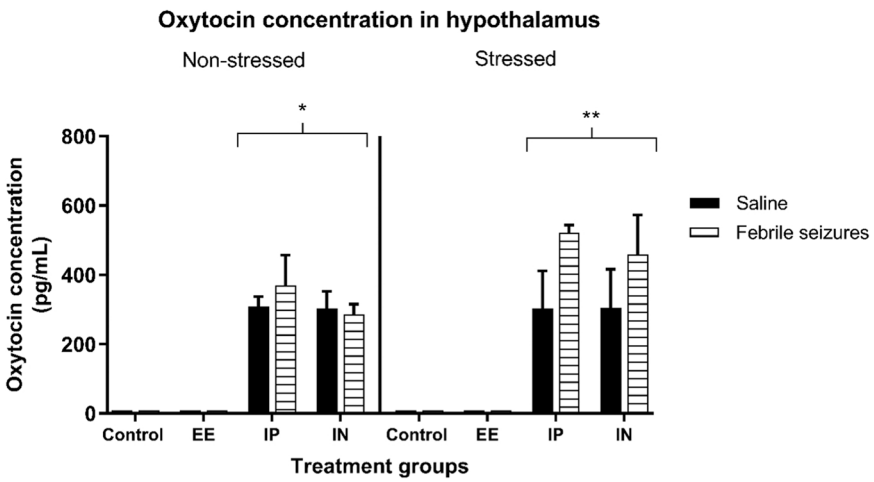

Fig. 5. : Graph showing oxytocin concentrations (pg/mL) in the hypothalamus. Eight subgroups were used for the non-stressed study while another 8 subgroups for the stressed study. Significant differences were observed between * (NS-C vs. NS-IP, $p < 0.0378$); * (NS-FS-NO vs. NS-FS-IP, $p < 0.0049$); * (NS-C vs. NS-IN, $p = 0.0435$); * (NS-FS-NO vs. NS-FS-IN, $p < 0.0744$) and * *(S-NO vs. S-IP, $p < 0.0437$); * *(S-FS-NO vs. S-FS-IP, $p < 0.0001$); * *(S-NO vs. S-IN, $p = 0.0419$); * *(S-FS-NO vs. S-FSIN, $p = 0.0002$). Data are presented as mean ± SEM ($n = 3$ rats per group).

et al., 2005, Mazarati, 2005, Heida et al., 2009, Wendorff and Zeman, 2011). This could subsequently cause an abnormally increased influx of Ca2 + , which then results in progressively increased neuronal firing, thus causing neuronal hyper-excitability which characterises the severe febrile convulsions (Viviani et al., 2003, Dubé et al., 2005, Mazarati, 2005, Heida et al., 2009, Wendorff and Zeman, 2011).

We further assessed whether an intervention via EE or OXT treatment (intraperitoneal and intranasal) would reduce the anxiety-like behaviour and plasma corticosterone concentrations seen in the prenatally stressed FS rat model. Our findings show that the treated animals spent a longer time exploring the light compartments of the light dark box, and open arms of the elevated plus maze, and that plasma corticosterone concentrations returned to baseline levels similar to that of the non-stressed control group. This suggests that exposure of prenatally stressed rats to an enriched environment or OXT administration during early postnatal development has the potential to reduce anxiety-like behaviour and HPA axis reactivity thereby reversing the increased basal plasma corticosterone concentrations caused by prenatal stress. Our finding agrees with previous studies such as that conducted by Li *et. al.* (2012) and Pascual *et. al.* (2015) where reduced anxiety-like behaviour was reported in prenatally stressed rats exposed to EE, since the rats spent an increased amount of time in the central and open arms, respectively, of the elevated plus maze (Li et al., 2012; Pascual et al., 2015). This suggests a more exploratory behaviour since the preference for the closed arms is a sign of need for security and reveals anxiety traits (Padurariu et al., 2017). Ring *et. al.* (2006) showed that both central and systemic OXT administration resulted in an anxiolytic effect where OXT treated mice showed an increased number of punished crossings in the four-plate test and a greater amount of time in the open quadrant of the elevated zero maze (Ring et al., 2006). Previous studies have also reported reduced plasma corticosterone concentrations following exposure to an enriched environment (Francis et al., 2002; Morley-Fletcher et al., 2003). This could be due to the fact that an enriched environment has been previously shown to increase serotonin receptor (5-HT1A) mRNA and receptor binding in the hippocampus (Rasmuson et al., 1998). This receptor binds the neurotransmitter serotonin and mediates inhibitory neurotransmission in the central nervous system (Jacobs and Azmitia, 1992). Interestingly, one of the fundamental effects of antidepressant medications is the enhancement of hippocampal 5-HT1A receptor binding (Blier, De Montigny, 1999; Piñeyro and Blier, 1999). These medications are known to dampen HPA axis activity and fear responses to stress (Blier, De Montigny, 1999; Piñeyro and Blier, 1999; Francis et al., 2002). It has also been shown that antidepressant medications attenuate the effect of prenatal stress on the HPA axis response to stress (Szymańska et al., 2009). Therefore, it is possible that both antidepressant medications and EE serve to dampen the activity of the HPA axis through effects on the 5-HT1A receptor system in the hippocampus. Notably, serotonin induces the secretion of endogenous OXT, as shown by previous studies where a serotonin agonist was capable of stimulating OXT production (Van De Kar et al., 1998). Since the OXTergic system serves to dampen the activity of the HPA axis under normal circumstances (Heinrichs et al., 2003; Olausson et al., 2003; Uvnas-Moberg and Petersson, 2005; Lee et al., 2007; Cardoso et al., 2013; Rault et al., 2013), and previous studies have shown that offspring exposed to prenatal stress have less OXT- producing neurons in the hypothalamus than those who were not exposed (de Souza et al., 2013, Wang et al., 2018), it could also be possible that EE reverses the effects of prenatal stress by the indirect stimulation of endogenous OXT production, through the effects of serotonin, thereby reversing the hyperactivity of the HPA axis caused by prenatal stress. Studies also showed reduced plasma corticosterone concentrations following OXT administration (Windle et al., 1997; Petersson et al., 1999; Olausson et al., 2003; Windle et al., 2004). This could be due to a sustained enhancement of α2 -adrenoceptor function in the nucleus of the solitary tract (NTS) and hypothalamus of prenatally stressed rats as well as in other brain regions in rats following treatment with OXT (Petersson et al., 1998; Día Z-Cabiale et al., 2000; Olausson et al., 2003). The sympathetic nervous system plays a role in an organism's stress response, particularly by releasing epinephrine and norepinephrine from the adrenal medulla of the adrenal glands, which then also plays a role (in addition to cortisol) in the "fight or flight" stress response (Guest et al., 2013). The epinephrine and norepinephrine also increase the activity of the HPA axis (Stephens and Wand, 2012; Guest et al., 2013). The α2 -adrenoceptor functions in inhibiting the release of norepinephrine (Cardiovascular Physiology et al.,) which would subsequently suppress the stress response and decrease HPA axis activity (since neurons of the NTS relay sensory information to the PVN, which subsequently regulates the HPA axis) (Smith and Vale, 2006). Therefore, this persistent enhancement of α2 -adrenoceptor function following postnatal treatment with OXT is of importance for the control of the autonomic nervous system and the HPA axis (Olausson et al., 2003; Smith and Vale, 2006). This could explain the decreased activity of the HPA axis and subsequent decrease in corticosterone levels (Olausson et al., 2003). Alternatively, since it's been shown that prenatally stressed rats have less endogenous opioid activity (Weinstock, 1997), and OXT exerts many of its effects by stimulating endogenous opioid release (Panksepp et al., 1997), the anti-stress effects observed following OXT treatment could be indirectly due to the action of OXT-induced endogenous opioids on the stress response. This is because under normal circumstances, endogenous opioids act by attenuating or terminating the stress response (Drolet et al., 2001).

We investigated both administration routes for OXT (systemic-

intraperitoneal or central-intranasal) to determine if there was a difference in its effectiveness since there is a possibility that the site of action could be different for either route of administration because the peripheral and central systems are separated by the blood brain barrier (Talegaonkar and Mishra, 2004; Ring et al., 2006). The behavioural results from both the light dark box and elevated plus maze indicate that OXT treatment results in anxiolytic effects through both administration routes but tended to be more potent when OXT was administered centrally (intranasally).

Finally, our findings also show that OXT administration - but not EE - was able to increase endogenous OXT secretion, as shown by the increased OXT concentrations in the hypothalamus. Unfortunately, the hypothalamic OXT concentrations for the control and enrichment groups are negative which could possibly be due to the detection limit of the ELISA kit used not being low enough to detect these concentrations. The kit sensitivity/ minimum detectable dose of OXT is 9.375 pg/mL, with a detection range of 15.625–1000 pg/mL. This could suggest that the hypothalamic OXT concentrations in these groups were below 9.375 pg/mL which is significantly lower than that of the OXT treated groups. Our findings are similar to that of previous studies where it was shown that exogenous intraperitoneal injection of OXT activated neurons in the paraventricular nucleus of the hypothalamus to release endogenous OXT and exerted metabolic effects similar to central OXT administration (Zhang and Cai, 2011; Carson et al., 2010). Following systemic OXT administration, only a small fraction crosses the blood brain barrier (Ermisch et al., 1985; Landgraf et al., 1979; Mens et al., 1983), which could subsequently stimulate the release of endogenous hypothalamic OXT via the autocrine feedforward system of OXT neurons (Moos et al., 1984; Rossoni et al., 2008). This is because hypothalamic neurons that release OXT contain several auto-receptors for the OXT they produce, therefore a small amount of OXT can activate this population of neurons into coordinated activity in a positive feedback mechanism (Rossoni et al., 2008). Since it was shown that this reflex can be facilitated by injection of tiny amounts of OXT into the hypothalamus (Rossoni et al., 2008), the fraction of OXT that could be crossing the blood brain barrier following OXT administration, could be entering the hypothalamus and having the same effect. This effect could be true for both administration routes: intraperitoneal (systemic) and intranasal (central), although it seems more potent in the intranasal groups. This could be due to higher amounts of OXT being able to cross the blood brain barrier following intranasal administration compared to intraperitoneal administration (Talegaonkar and Mishra, 2004).

Our results show that both oxytocin administration and exposure to an enriched environment were successful in attenuating the increased basal corticosterone levels and anxiety-like behaviour seen in the prenatally stressed febrile seizure rat model, although the effects of oxytocin administration were more potent. Therefore, oxytocin administration during early postnatal development shows great potential in reversing the effects of prenatal stress and its subsequent exacerbation of febrile seizures. Such results could help prenatally stressed children who have been affected by the extremely common and agonizing febrile seizures, to overcome or prevent the resulting neurological ramifications. This may subsequently support normal neurological function to promote a healthier life. Furthermore, since current febrile seizure treatments only treat the seizure and not the neurological ramifications, oxytocin or an enriched environment could be used as an adjunct with the current febrile seizure treatment regimes, so that both the seizure and its subsequent neurological ramifications are treated. This will decrease the susceptibility of prenatally stressed offspring to developing febrile seizures or even if they do develop these seizures, the severity will not be as exaggerated. This could ultimately reduce the chances of these seizures leading to cognitive and behavioural deficits later in life, as well as reducing the susceptibility to epilepsy. To the best of our knowledge, this is the first study to explore the effects of oxytocin administration and exposure to an enriched environment on basal corticosterone levels and anxiety-like behaviour in a prenatally stressed febrile seizure rat model, let alone on human subjects. However, a previous study by Munesue et al. (2016) which investigated the effects of intranasal oxytocin treatment in adolescents and adults with autism spectrum disorder and epilepsy on core symptoms of social deficits, reported seizures in two participants. The first patient's seizure, which occurred during the follow-up phase, was proposed to have been caused by non-adherence to his anti-epileptic drug. Although the second patient exhibited seizures throughout the intervention and control phases of the crossover study, even though his epilepsy had previously been well controlled; and the patient's last seizure had been 7 years prior to the trial and adherence to both oxytocin and his anti-epileptic drugs were found to be excellent during the course of the trial, it is not known what the effect of early postnatal treatment with intranasal oxytocin would have been on these seizures, neither is it known whether or not this participant was exposed to prenatal stress. Furthermore, none of the seven patients who had epilepsy that were recruited for this study suffered seizure attacks during the course of the study, except for this aforementioned patient (Munesue et al., 2016). Other oxytocin administration experiments on human subjects have also highlighted oxytocin as an anti-stress hormone and show attenuated anxiety and cortisol reactivity after intranasal administration, in particular when confronted with social stressors (Cardoso et al., 2013a, 2013b; Kubzansky et al., 2012; Linnen et al., 2012). However, no studies have particularly assessed the effects of early postnatal treatment with intranasal oxytocin in prenatally stressed children on anxiety and cortisol reactivity, let alone whether this has an effect on seizure exacerbation. Therefore, future studies need to explore the long-term effects of early postnatal oxytocin administration or exposure to an enriched environment on seizure severity as well as basal corticosterone levels and anxiety-like behaviour in prenatally stressed children with febrile seizures.

Additionally, the effect of EE or OXT administration during early postnatal development on the susceptibility to any continued seizures later on should be considered for future studies. This is because previous studies have shown that EE significantly reduced seizure episodes and seizure duration in epileptic rats, as well as alleviating depression (Vrinda et al., 2017). It was also shown that OXT may have anti-convulsant effects where pentylenetetrazol (PTZ) - induced seizures were effectively prevented following OXT administration (Erbas et al., 2013). An antiepileptic effect of OXT was also shown following intra-hippocampal microinjections of OXT and diazepam, where OXT increased the antiepileptic effect of diazepam (Erfanparast et al., 2017). This brings forth the possibility that OXT or an enriched environment could be used as an adjunct with the current FS treatment regimes, so that both the seizure and its subsequent neurological ramifications are treated.

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Declaration of Competing Interest

The authors declare no personal or financial conflicting interests.

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References

Blier, P., De Montigny, C., 1999. Serotonin and drug-induced therapeutic responses in major depression, obsessive–compulsive and panic disorders. Neuropsychopharmacology 21, 91S–98S.

Blume, A., Bosch, O.J., Miklos, S., Torner, L., Wales, L., Waldherr, M., Neumann, I.D., 2008. Oxytocin reduces anxiety via ERK1/2 activation: local effect within the rat hypothalamic paraventricular nucleus. Eur. J. Neurosci. 27, 1947–1956.
Bourin, M., Hascoet, M., 2003. The mouse light/dark box test. Eur. J. Pharm. 463, 55–65.
Buitelaar, J.K., Huizink, A.C., Mulder, E.J., De Medina, P.G., Visser, G.H., 2003. Prenatal stress and cognitive development and temperament in infants. Neurobiol. Aging 24 (Suppl 1), S53–S60 discussion S67-8.
Cardiovascular Physiology, R.E., Arnold Publishers, Levick, J.R., CHAPTER 14.1, SYMPATHETIC VASOCONSTRICTOR NERVES.
Cardoso, C., Ellenbogen, M.A., Orlando, M.A., Bacon, S.L., Joober, R., 2013. Intranasal oxytocin attenuates the cortisol response to physical stress: a dose-response study. Psychoneuroendocrinology 38, 399–407.
Carson, D.S., Hunt, G.E., Guastella, A.J., Barber, L., Cornish, J.L., Arnold, J.C., Boucher, A.A., Mcgregor, I.S., 2010. Systemically administered oxytocin decreases methamphetamine activation of the subthalamic nucleus and accumbens core and stimulates oxytocinergic neurons in the hypothalamus. Addict. Biol. 15, 448–463.
Cassim, S., Qulu, L., Mabandla, M.V., 2015. Prenatal stress and early life febrile convulsions compromise hippocampal genes MeCP2/REST function in mid-adolescent life of Sprague- Dawley rats. Neurobiol. Learn Mem. 125, 195–201.
Charil, A., Laplante, D.P., Vaillancourt, C., King, S., 2010. Prenatal stress and brain development. Brain Res. Rev. 65, 56–79.
Davis, E.P., Glynn, L.M., Waffarn, F., Sandman, C.A., 2011. Prenatal maternal stress programs infant stress regulation. J. Child Psychol. Psychiatry, Allied Discip. 52, 119–129.
Kloet, D.E., E. R, Claessens, S.E., Kentrop, J., 2014. Context modulates outcome of perinatal glucocorticoid action in the brain. Front. Endocrinol. (Lausanne) 5, 100.
Souza, D.E., Centenaro, M.A., Menegotto, L.A., Henriques, P.R., Bonini, T.P., J., Achaval, M., Lucion, A.B., 2013. Prenatal stress produces social behavior deficits and alters the number of oxytocin and vasopressin neurons in adult rats. Neurochem. Res. 38, 1479–1489.
Diá Z-Cabiale, Z., Petersson, M., Narváez, J.A., Uvnäs-Moberg, K., Fuxe, K., 2000. Systemic oxytocin treatment modulates α2-adrenoceptors in telencephalic and diencephalic regions of the rat. Brain Res. 887, 421–425.
Drolet, G., Dumont, É.C., Gosselin, I., Kinkead, R., Laforest, S., Trottier, J.-F., 2001. Role of endogenous opioid system in the regulation of the stress response. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 25, 729–741.
Dube, C., Vezzani, A., Behrens, M., Bartfai, T., Baram, T.Z., 2005. Interleukin-1beta contributes to the generation of experimental febrile seizures. Ann. Neurol. 57, 152–155.
Dubé, C.M., Brewster, A.L., Baram, T.Z., 2009. Febrile seizures: Mechanisms and relationship to epilepsy. Brain Dev. 31, 366–371.
Emack, J., Matthews, S.G., 2011. Effects of chronic maternal stress on hypothalamo-pituitaryadrenal (HPA) function and behavior: no reversal by environmental enrichment. Horm. Behav. 60, 589–598.
Erbas, O., Yılmaz, M., Korkmaz, H.A., Bora, S., Evren, V., Peker, G., 2013. Oxytocin inhibits pentylentetrazol-induced seizures in the rat. Peptides 40, 141–144.
Erfanparast, A., Tamaddonfard, E., Henareh-Chareh, F., 2017. Intra-hippocampal microinjection of oxytocin produced antiepileptic effect on the pentylenetetrazol-induced epilepsy in rats. Pharmacol. Rep. 69, 757–763.
Ermisch, A., Barth, T., Rühle, H., Skopkova, J., Hrbas, P., Landgraf, R., 1985. On the bloodbrain barrier to peptides: accumulation of labelled vasopressin, DesGlyNH2-vasopressin and oxytocin by brain regions. Endocrinol. Exp. 19, 29–37.
File, S.E., 1993. The interplay of learning and anxiety in the elevated plus-maze. Behav. Brain Res 58, 199–202.
Francis, D.D., Diorio, J., Plotsky, P.M., Meaney, M.J., 2002. Environmental enrichment reverses the effects of maternal separation on stress reactivity. J. Neurosci. 22, 7840–7843.
Frank, M.G., Watkins, L.R., Maier, S.F., 2013. Stress-induced glucocorticoids as a neuroendocrine alarm signal of danger. Brain, Behav., Immun. 33, 1–6.
Frank, M.G., Watkins, L.R., Maier, S.F., 2015. The permissive role of glucocorticoids in neuroinflammatory priming: mechanisms and insights. Curr. Opin. Endocrinol., Diabetes, Obes. 22, 300.
Freeman, J.M., 1980. Febrile seizures: a consensus of their significance, evaluation, and treatment. Pediatrics 66, 1009-1009.
Gatti, S., Beck, J., Fantuzzi, G., Bartfai, T., Dinarello, C.A., 2002. Effect of interleukin-18 on mouse core body temperature. Am. J. Physiol. -Regul., Integr. Comp. Physiol. 282, R702–R709.
Guest, F.L., Martins-De-Souza, D., Rahmoune, H., Bahn, S., Guest, P.C., 2013. The effects of stress on hypothalamic-pituitary-adrenal (HPA) axis function in subjects with schizophrenia. Arch. Clin. Psychiatry (São Paulo) 40, 20–27.
Harris, A., Seckl, J., 2011. Glucocorticoids, prenatal stress and the programming of disease. Horm. Behav. 59, 279–289.
Heida, J.G., Boissé, L., Pittman, Q.J., 2004. Lipopolysaccharide-induced febrile convulsions in the rat: short-term sequelae. Epilepsia 45, 1317–1329.
Heida, J.G., Moshe, S.L., Pittman, Q.J., 2009. The role of interleukin-1beta in febrile seizures. Brain Dev. 31, 388–393.
Heinrichs, M., Baumgartner, T., Kirschbaum, C., Ehlert, U., 2003. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. Biol. Psychiatry 54, 1389–1398.
Hubscher, C.H., Brooks, D.L., Johnson, J.R., 2005. A quantitative method for assessing stages of the rat estrous cycle. Biotech. Histochem 80, 79–87.
Hwang, G., Kang, H.S., Park, S.Y., Han, K.H., Kim, S.H., 2015. Predictors of unprovoked seizure after febrile seizure: short-term outcomes. Brain Dev. 37, 315–321.
Jacobs, B.L., Azmitia, E.C., 1992. Structure and function of the brain serotonin system. Physiol. Rev. 72, 165–229.
Kariuki, S.M., Abubakar, A., Stein, A., Marsh, K., Newton, C.R., 2017. Prevalence, causes, and behavioral and emotional comorbidities of acute symptomatic seizures in Africa: A critical review. Epilepsia Open 2, 8–19.
Kubzansky, L.D., Mendes, W.B., Appleton, A.A., Block, J., Adler, G.K., 2012. A heartfelt response: oxytocin effects on response to social stress in men and women. Biol. Psychol. 90 (1), 1–9.
Landgraf, R., Ermisch, A., Hess, J., 1979. Indications for a brain uptake of labelled vasopressin and ocytocin and the problem of the blood-brain barrier. Endokrinologie 73, 77–81.
Lee, P.R., Brady, D.L., Shapiro, R.A., Dorsa, D.M., Koenig, J.I., 2007. Prenatal stress generates deficits in rat social behavior: Reversal by oxytocin. Brain Res 1156, 152–167.
Li, M., Wang, M., Ding, S., Li, C., Luo, X., 2012. Environmental enrichment during gestation improves behavior consequences and synaptic plasticity in hippocampus of prenatal-stressed offspring rats. Acta Histochem. Et. Cytochem. 45, 157–166.
Linnen, A.M., Ellenbogen, M.A., Cardoso, C., Joober, R., 2012. Intranasal oxytocin and salivary cortisol concentrations during social rejection in university students. Stress 15 (4), 393–402.
Mazarati, A.M., 2005. Cytokines: a link between fever and seizures. Epilepsy Curr. 5, 169–170.
Mccreary, J.K., Metz, G.A.S., 2016. Environmental enrichment as an intervention for adverse health outcomes of prenatal stress. Environ. Epigenetics 2 dvw013-dvw013.
Mens, W.B.J., Witter, A., VAN Wimersma Greidanus, T.B., 1983. Penetration of neurohypophyseal hormones from plasma into cerebrospinal fluid (CSF): Half-times of disappearance of these neuropeptides from CSF. Brain Res. 262, 143–149.
Moos, F., Freund-Mercier, M., Guerne, Y., Guerne, J., Stoeckel, M., Richard, P., 1984. Release of oxytocin and vasopressin by magnocellular nuclei in vitro: specific facilitatory effect of oxytocin on its own release. J. Endocrinol. 102, 63.
Morley-Fletcher, S., Rea, M., Maccari, S., Laviola, G., 2003. Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats. Eur. J. Neurosci. 18, 3367–3374.
Munesue, T., Nakamura, H., Kikuchi, M., et al., 2016. Oxytocin for male subjects with autism Spectrum disorder and comorbid intellectual disabilities: A randomized pilot study. Front. Psychiatry 7, 2.
Nelson, J.F., Felicio, L.S., Randall, P.K., Sims, C., Finch, C.E., 1982. A longitudinal study of estrous cyclicity in aging C57BL/6J mice: I. Cycle frequency, length and vaginal cytology. Biol. Reprod. 27, 327–339.
Ojewole, J.A., 2008. Anticonvulsant effect of Rhus chirindensis (Baker F.) (Anacardiaceae) stem-bark aqueous extract in mice. J. Ethnopharmacol. 117, 130–135.
Olausson, H., Uvnas-Moberg, K., Sohlstrom, A., 2003. Postnatal oxytocin alleviates adverse effects in adult rat offspring caused by maternal malnutrition. Am. J. Physiol. Endocrinol. Metab. 284, E475–E480.
Padurariu, M., Antioch, I., Balmus, I., Ciobica, A., Heba, E.-L., Kamel Mervet, M., 2017. Describing some behavioural animal models of anxiety and their mechanistics. Oxidative stress and oxytocin relevance? Int. J. Vet. Sci.
Panksepp, J., Nelson, E., Bekkedal, M., 1997. Brain Systems for the Mediation of Social Separation- Distress and Social-Reward Evolutionary Antecedents and Neuropeptide Intermediaries a. Ann. N. Y. Acad. Sci. 807, 78–100.
Pascual, R., Valencia, M., Bustamante, C., 2015. Purkinje cell dendritic atrophy induced by prenatal stress is mitigated by early environmental enrichment. Neuropediatrics 46, 037–043.
Petersson, M., Hulting, A.-L., Uvnäs-Moberg, K., 1999. Oxytocin causes a sustained decrease in plasma levels of corticosterone in rats. Neurosci. Lett. 264, 41–44.
Petersson, M., Uvnas-Moberg, K., Erhardt, S., Engberg, G., 1998. Oxytocin increases locus coeruleus alpha 2-adrenoreceptor responsiveness in rats. Neurosci. Lett. 255, 115–118.
Piñeyro, G., Blier, P., 1999. Autoregulation of serotonin neurons: role in antidepressant drug action. Pharmacol. Rev. 51, 533–591.
Qulu, L., Daniels, W.M., Mabandla, M.V., 2012. Exposure to prenatal stress enhances the development of seizures in young rats. Metab. Brain Dis. 27, 399–404.
Qulu, L., Daniels, W.M., Mabandla, M.V., 2015. Exposure to prenatal stress has deleterious effects on hippocampal function in a febrile seizure rat model. Brain Res 1624, 506–514.
Rasmuson, S., Olsson, T., Henriksson, B.G., Kelly, P.A., Holmes, M.C., Seckl, J.R., Mohammed, A.H., 1998. Environmental enrichment selectively increases 5-HT1A receptor mRNA expression and binding in the rat hippocampus. Mol. Brain Res. 53, 285–290.
Rault, J.-L., Mack, L.A., Carter, C.S., Garner, J.P., Marchant-Forde, J.N., Richert, B.T., Lay, D.C., 2013. Prenatal stress puzzle, the oxytocin piece: Prenatal stress alters the behaviour and autonomic regulation in piglets, insights from oxytocin. Appl. Anim. Behav. Sci. 148, 99–107.
Riazi, K., Galic, M.A., Pittman, Q.J., 2010. Contributions of peripheral inflammation to seizure susceptibility: Cytokines and brain excitability. Epilepsy Res. 89, 34–42.
Ring, R.H., Malberg, J.E., Potestio, L., Ping, J., Boikess, S., Luo, B., Schechter, L.E., Rizzo, S., Rahman, Z., Rosenzweig-Lipson, S., 2006. Anxiolytic-like activity of oxytocin in male mice: behavioral and autonomic evidence, therapeutic implications. Psychopharmacology 185, 218–225.
Rossoni, E., Feng, J., Tirozzi, B., Brown, D., Leng, G., Moos, F., 2008. Emergent synchronous bursting of oxytocin neuronal network. PLoS Comput. Biol. 4, e1000123.
Smith, S.M., Vale, W.W., 2006. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. Dialog-. Clin. Neurosci. 8, 383–395.
Sorrells, S.F., Caso, J.R., Munhoz, C.D., Sapolsky, R.M., 2009. The Stressed CNS: When Glucocorticoids Aggravate Inflammation. Neuron 64, 33–39.

Spencer, S.J., Heida, J.G., Pittman, Q.J., 2005. Early life immune challenge–effects on behavioural indices of adult rat fear and anxiety. Behav. Brain Res 164, 231–238.
Stephens, M.A., Wand, G., 2012. Stress and the HPA axis: role of glucocorticoids in alcohol dependence. Alcohol Res 34, 468–483.
Szymańska, M., Budziszewska, B., Jaworska-Feil, L., Basta-Kaim, A., Kubera, M., Leśkiewicz, M., Regulska, M., Lasoń, W., 2009. The effect of antidepressant drugs on the HPA axis activity, glucocorticoid receptor level and FKBP51 concentration in prenatally stressed rats. Psychoneuroendocrinology 34, 822–832.
Talegaonkar, S., Mishra, P., 2004. Intranasal delivery: An approach to bypass the blood brain barrier. Indian J. Pharmacol. 36, 140.
Uvnas-Moberg, K., Petersson, M., 2005. [Oxytocin, a mediator of anti-stress, well-being, social interaction, growth and healing]. Z. Psychosom. Med Psychother. 51, 57–80.
Van De Kar, L.D., Levy, A.D., Li, Q., Brownfield, M.S., 1998. A comparison of the oxytocin and vasopressin responses to the 5-HT1A agonist and potential anxiolytic drug alnespirone (S- 20499). Pharmacol. Biochem. Behav. 60, 677–683.
Van Praag, H., Kempermann, G., Gage, F.H., 2000. Neural consequences of environmental enrichment. Nat. Rev. Neurosci. 1, 191–198.
Vega-Rivera, N.M., Ortiz-López, L., Gómez-Sánchez, A., Oikawa-Sala, J., Estrada-Camarena, E.M., Ramírez-Rodríguez, G.B., 2016. The neurogenic effects of an enriched environment and its protection against the behavioral consequences of chronic mild stress persistent after enrichment cessation in six-month-old female Balb/C mice. Behav. Brain Res. 301, 72–83.
Viviani, B., Bartesaghi, S., Gardoni, F., Vezzani, A., Behrens, M., Bartfai, T., Binaglia, M., Corsini, E., Di Luca, M., Galli, C., 2003. Interleukin-1β enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. J. Neurosci. 23, 8692–8700.
Vrinda, M., Sasidharan, A., Aparna, S., Srikumar, B.N., Kutty, B.M., Shankaranarayana Rao, B.S., 2017. Enriched environment attenuates behavioral seizures and depression in chronic temporal lobe epilepsy. Epilepsia 58, 1148–1158.
Wang, T., Shi, C., Li, X., Zhang, P., Liu, B., Wang, H., Wang, Y., Yang, Y., Wu, Y., Li, H., Xu, Z.-Q.D., 2018. Injection of oxytocin into paraventricular nucleus reverses depressive-like behaviors in the postpartum depression rat model. Behav. Brain Res. 336, 236–243.
Wang, Y., Aun, R., Tse, F.L., 1998. Brain uptake of dihydroergotamine after intravenous and nasal administration in the rat. Biopharm. Drug Dispos. 19, 571–575.
Waruiru, C., Appleton, R., 2004. Febrile seizures: an update. Arch. Dis. Child. 89, 751–756.
Weinstock, M., 1997. Does Prenatal Stress Impair Coping and Regulation of Hypothalamic-Pituitary- Adrenal Axis? Neurosci. Biobehav. Rev. 21, 1–10.
Wendorff, J., Zeman, K., 2011. Immunology of febrile seizures. Neurol. Dziecieca 20, 40–46.
Windle, R.J., Kershaw, Y.M., Shanks, N., Wood, S.A., Lightman, S.L., Ingram, C.D., 2004. Oxytocin attenuates stress-induced c-fos mRNA expression in specific forebrain regions associated with modulation of hypothalamo-pituitary-adrenal activity. J. Neurosci. 24, 2974–2982.
Windle, R.J., Shanks, N., Lightman, S.L., Ingram, C.D., 1997. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. Endocrinology 138, 2829–2834.
Zhang, G., Cai, D., 2011. Circadian intervention of obesity development via resting-stage feeding manipulation or oxytocin treatment. Am. J. Physiol. Endocrinol. Metab. 301, E1004–E1012.