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Research Paper

Prolonged efavirenz exposure reduces peripheral oxytocin and vasopressin comparable to known drugs of addiction in male Sprague Dawley rats

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ABSTRACT

Introduction: Several drugs of abuse (DOA) are capable of modulating neurohypophysial hormones, such as oxytocin (OT) and vasopressin (VP), potentially resulting in the development of psychological abnormalities, such as cognitive dysfunction, psychoses, and affective disorders. Efavirenz (EFV), widely used in Africa and globally to treat HIV, induces diverse neuropsychiatric side effects while its abuse has become a global concern. The actions of EFV may involve neurohypophysial system (NS) disruption like that of known DOA. This study investigated whether sub-chronic EFV exposure, at a previously-determined rewarding dose, alters peripheral OT and VP levels versus that of a control, Δ^9-tetrahydrocannabinol (Δ^9-THC), methamphetamine (MA) and cocaine.
Materials and methods: To simulate the conditions under which reward-driven behavior had previously been established for EFV, male Sprague Dawley rats ($n = 16$/exposure) received intraperitoneal vehicle (control) or drug administration across an alternating sixteen-day dosing protocol. Control administration (saline/olive oil; 0.2 ml) occurred on odd-numbered and drug administration (EFV: 5 mg/kg, Δ^9-THC: 0.75 mg/kg, MA: 1 mg/kg, or cocaine: 20 mg/kg) on even-numbered days followed by euthanasia, trunk blood collection and plasma extraction for neuropeptide assay. Effect of drug exposure on peripheral OT and VP levels was assessed versus controls and quantified using specific ELISA kits. Statistical significance was determined by Kruskal-Wallis ANOVA, with $p < 0.05$. Ethics approval: NWU-00291-17-A5.
Results: Delta-9-THC reduced OT and VP plasma levels ($p < 0.0001$, $p = 0.0141$; respectively), cocaine reduced plasma OT ($p = 0.0023$), while MA reduced plasma VP levels ($p = 0.0001$), all versus control. EFV reduced OT and VP plasma levels ($p < 0.0001$; OT and VP) versus control, and similar to Δ^9-THC.
Conclusion: EFV markedly affects the NS in significantly reducing *both* plasma OT and VP equivalent to DOA. Importantly, EFV has distinct effects on peripheral OT and VP levels when assessed within the context of drug dependence. The data highlights a possible new mechanism underlying previously documented EFV-induced effects in rats, and whereby EFV may induce neuropsychiatric adverse effects clinically; also providing a deeper understanding of the suggested abuse-potential of EFV.

Abbreviations: Δ^9-THC, delta-9-tetrahydrocannabinol; 5-HT, 5-hydroxytryptamine (serotonin); Ach, acetylcholine; ADH, antidiuretic hormone; AEA, N-arachidonoylethanolamine (anandamide); ANOVA, *one-way* analysis of variance; ARRIVE, animal research: reporting of in vivo experiments (guidelines); ARV, antiretroviral; cART, combined antiretroviral therapy; CB, cannabinoid; CNS, central nervous system; CPP, conditioned place preference; DA, dopamine; DAT, dopamine transporter; DOA's, drug(s) of abuse; ECS, endocannabinoid system; EFV, efavirenz; ELISA, enzyme-linked immunosorbent assay; GABA, gamma-aminobutyric acid; Glu, glutamate; HIV, human immunodeficiency virus; HNS, hypothalamic neurohypophysial system; HPA, hypothalamic-pituitary-adrenal (axis); IV, intravenous; IP, intraperitoneal; M, muscarinic; MA, methamphetamine; MAO, monoamine oxidase; NAc, nucleus accumbens; NE, norepinephrine; NO, nitric oxide; NPAE, neuropsychiatric adverse effect; OT, oxytocin; OTR, oxytocin receptor; PND, postnatal day; PVN, paraventricular nucleus; SC, subcutaneous; SD, Sprague Dawley (rat); SEM, standard error of the mean; SERT, serotonin transporter; SON, supraoptic nucleus; VMAT, vesicular monoamine transporter; VP, vasopressin; VPR, vasopressin receptor.

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1. Introduction

Efavirenz (EFV) is an antiretroviral (ARV) drug used in treating human immune-deficiency virus (HIV) infection (Dalwadi et al., 2016). At a standard dose (600 mg per day) EFV is known to cause neuropsychiatric adverse effects (NPAEs), such as anxiety, depression, and psychosis (Dalwadi et al., 2018). This action is attributed to its ability to effectively reach the brain and exert central nervous system (CNS) effects (Decloedt et al., 2015). The neuropsychological profile and the underlying actions associated with EFV-induced NPAEs are, however, not yet fully understood (see Dalwadi et al., 2018 for review).

Due to its alleged euphoric or hallucinogenic effects and potential to enhance such sensations in the presence of recreational/dependence-forming drugs, EFV has been associated with reports of misuse in combination with cannabis, heroin, amphetamines, and cocaine (Bracchi et al., 2015; also see Dalwadi et al., 2018 for review). The combination of EFV with other drugs of abuse (DOA), especially cannabis, in a cocktail known as *Whoonga* or *Nyaope*, is especially noteworthy (Gatch et al., 2013; Grelotti et al., 2014; Morris, 2014; Rough et al., 2014; also reviewed by Dalwadi et al., 2018). Both acute and sub-chronically administered EFV (5 mg/kg, IP) stimulates reward-driven behavior, as well as increases dopamine (DA) levels in mesolimbic reward structures, such as the striatum (Möller et al., 2018). When combined with Δ^9-tetrahydrocannabinol (THC; 0.75 mg/kg) the rewarding effects are maintained, with the combination also prompting hedonic behavior in rats. Furthermore, EFV plus Δ^9-THC led to a greater increase in striatal DA compared to Δ^9-THC alone (Möller et al., 2018).

Monoamines like DA have taken center stage in addiction research (see Uhl et al., 2019 for review). However, the involvement of the hypothalamic neurohypophysial system (HNS) and its associated neurohormones, oxytocin (OT) and vasopressin (VP), remains to be fully explicated. The relationship between drug dependence and the HNS has been suggested to involve a bi-directional interaction between dopaminergic and oxytocinergic neurocircuitry within various brain regions (Peris et al., 2017; also see Baskerville and Douglas, 2010; Knobloch and Grinevich, 2014 for review). Importantly, changes in OT and VP are associated with DOA (Wilkinson and Brown, 2015), while reward/incentive-driven behaviors are said to involve dopaminergic *and* oxytocinergic communication in the brain (Bates et al., 2018; Peris et al., 2017). Moreover, OT has therapeutic potential in the treatment of substance abuse disorders (reviewed by Leong et al., 2018; Lee et al., 2016), while also being advocated as a biomarker for drug dependence (Butovsky et al., 2006; see Bowen and Neumann, 2017; Buisman-Pijlman et al., 2014 for review). Vasopressin, on the other hand, appears to be less valuable in this regard (Rodríguez-Borrero et al., 2010). Both OT and VP are associated with the manifestation of different mammalian social, sexual, and emotional behaviors, as well as cognitive processes (for review, see Neumann and Landgraf, 2012). Generally, VP is linked to negative behavioral responses (e.g. depression, anxiety, and behavioral defensiveness) and learning and memory deficits while OT has the opposite effects (as reviewed by Neumann and Landgraf, 2012; Stoop, 2012). Furthermore, the two neurohormones act in juxtaposition to each other with regards to drug-induced behavioral modifications (see Buisman-Pijlman et al., 2014; Stoop, 2012 for review). Importantly, both neuropeptides interact with other signaling systems critical in reward processing and addiction, such as DA, serotonin (5-HT) and norepinephrine (NE), acetylcholine (ACh), gamma-aminobutyric acid (GABA), glutamate (Glu), nitric oxide (NO), opioids and the endocannabinoid system (ECS) (as reviewed by Wilkinson and Brown, 2015; Zanos et al., 2018).

Seeing that a significant amount of literature provides evidence of DOA-induced changes in the HNS, and associated oxytocinergic and vasopressogenic systems (as reviewed by Buisman-Pijlman et al., 2014; Godino and Renard, 2018; Leong et al., 2018; Wilkinson and Brown, 2015), it is reasonable to hypothesize that EFV may induce comparable effects in these systems. Therefore, we sought to examine the effects of sub-chronic EFV exposure on peripheral OT and VP levels when administered at a dose and exposure regime previously demonstrated to stimulate reward-driven behavior in male Sprague Dawley rats (Möller et al., 2018). These effects were compared to test groups exposed to vehicle/control, MA, cocaine, and Δ^9-THC. We propose that EFV will bring about changes in plasma OT and VP, as do MA, cocaine, and Δ^9-THC, but with important differences.

2. Materials and methods

2.1. Animals

Ninety-six (96) male Sprague Dawley (SD) rats (7–8 weeks of age) were used in this study and provided by the NWU Vivarium (SAVC reg. number FR15/13458, SANAS GLP compliance number G0019), a division of the Pre-Clinical Drug Development Platform of the NWU. Importantly, female SD rats were excluded from the study based on likely interference of the estrus cycle in the measured biological markers (Becker and Koob, 2016). The SD strain is frequently applied in preclinical research in investigating drug effects and neuropsychological disorders (Ellenbroek and Youn, 2016).

All animals were bred, housed, maintained and handled in the Vivarium according to the code of ethics for using animals in research, training, and testing in South Africa, with all aforementioned done per the national legislature. On postnatal day 21 (PND21) animals were weaned and randomized to their home cages (2–3 animals per cage) and designated exposure groups ($n = 16$ per exposure $\times$ nine groups). Animal well-being was monitored using specific monitoring sheets (Schoeman et al., 2017). Animals had access to food and water ad-libitum. All home cages were maintained under identical environmental conditions (22 $\pm$ 1 °C; 55 $\pm$ 10% humidity) and included environmental enrichment in the form of polyvinyl chloride pipes. A simulated 12 h light/dark cycle was applied (lights on 06:00 until 18:00; 350–400 lux white light) (Schoeman et al., 2017).

The study framework, ethical considerations, statistical analyses, and final report compilation were executed in-line with the ARRIVE guidelines (Kilkenny et al., 2010). Ethical authorization for all procedures was obtained before its initiation from the North-West University (NWU) animal research ethics committee, AnimCare (NHREC reg. number AREC-130913-015) (**NWU-00291-17-A5**).

2.2. Drugs and exposure protocol

EFV was kindly sponsored by Aspen Pharmaceuticals, Port Elizabeth, South Africa. Delta-9-THC and methamphetamine HCl were purchased from Sigma-Aldrich (Pretoria, South Africa) and cocaine HCl from Transpharm (Pty) Ltd (Pretoria, South Africa). Normal saline (0.9%) and pharmaceutical-grade olive oil were used as vehicles for drug dissolution and served as the corresponding vehicle controls, as described below.

All exposures were administered sub-chronically via the intraperitoneal (IP) route (Turner et al., 2011). Vehicle and/or drug administration took place each morning (between 07:00 and 10:00) for 16 days (PND53 until PND68) (Möller et al., 2018). Exposures alternated between vehicle (on odd-numbered days) and drug (on even-numbered days). This study design was performed following a previously validated EFV dosing protocol, used in behavioral conditioning (specifically the condition place preference (CPP) paradigm) shown to induce incentive behavior in rats (Malanga et al., 2007; Möller et al., 2018).

Both EFV (5 mg/kg) and Δ^9-THC (0.75 mg/kg) are lipophilic (Decloedt et al., 2015; Lundberg et al., 2005) and were therefore prepared in olive oil (vehicle; 0.2 ml) which, as stated, also served as their corresponding control (Möller et al., 2018). However, though it has no rewarding effects, it is worth mentioning that recent preclinical investigations suggest that high-quality commercial-grade (extra virgin) olive oil is capable of modulating both OT and VP activity (Sospedra

et al., 2015; Villarejo et al., 2015). That said, the focus of the latter studies was on the use of olive oil as a dietary supplement in the management of appetite and cardiovascular disease, respectively, and produced somewhat ambiguous results. MA (1 mg/kg) and cocaine (20 mg/kg) were prepared in saline, which also served as their corresponding control, and the solutions buffered using NaOH and 1 M glacial acetic acid (pH = 6.0–6.8) (Möller et al., 2018). The chosen doses for the various exposures are based on previous studies demonstrating their rewarding effects when screened using CPP testing (Braida et al., 2004; Möller et al., 2018; Nygard et al., 2015; Xu et al., 2016).

2.3. Bodyweight

Animals were weighed from PND21 until PND69. This was done to continuously monitor animal development, allow for familiarization toward human handling and to ensure normal healthy development across all exposure groups. Furthermore, daily weighing allowed for accurate drug-dose determination for each animal during the exposure period.

2.4. Plasma oxytocin (OT) and vasopressin (VP) analysis

On PND69 (between 07:00 and 10:00; no drug or vehicle exposure on the day) the animals were euthanized through decapitation without prior anesthesia and their trunk blood collected. Trunk blood was captured in pre-chilled 4 ml vacutainer tubes (SGVac) lined with an anticoagulant solution, K_2EDTA. Immediately thereafter the blood samples were centrifuged, the plasma (supernatant) extracted and OT and VP levels quantified using specific enzyme-linked immunosorbent assay (ELISA) kits (Elabscience® (www.elabscience.com) and Abcam® (www.abcam.com), respectively); performed as per manufacturer instructions. Research also demonstrates the clinical relevance of systemic neuropeptide fluctuations as potential indicators of psychological conditions (Quirin et al., 2011; Scantamburlo et al., 2007; also reviewed by Neumann and Landgraf, 2012). There is some preclinical evidence to suggest mirrored neuropeptide changes in central and peripheral systems (Nishioka et al., 1998; Sarnyai et al., 1992a, 1992b). Additionally, Baracz and Cornish (2016) proposed that plasma neuropeptide oscillations (viz. OT) may be indicative of drug (MA)-induced changes within the CNS, though not specific to a single locus (Buisman-Pijlman et al., 2014), which they coupled with findings from their earlier research (Baracz et al., 2016).

2.4.1. OT

Sample preparation and assays were performed as per the manufacturer instructions, with OT quantified in a microplate reader (SpectraMax ® Paradigm® Multi-Mode Microplate Reader, Molecular Devices, Inc.) set at an optical density of 450 nm. The data collected were quantified from a standard curve and expressed as pg/ml.

2.4.2. VP. Sample preparation

Before performing the ELISA kit procedure, vasopressin (Arg^8-vasopressin) extraction had to be done to produce concentrated samples for assay: 400 µl supernatant was extracted and pipetted into plastic Eppendorf® tubes followed by addition of 2 × volume (800 µl) ice-cold acetone to each sample. The aforementioned extraction procedure is an important *additional* step in the sample preparation due to the concern for low endogenous levels of innate Arg^8-Vasopressin being present in the plasma extracts. The samples were then processed as per the manufacturer's instructions.

2.4.3. ELISA kit procedure

All assays were performed as per the manufacturer's instructions, with VP quantified in a microplate reader set at an optical density of 450 nm. The data collected were quantified from a standard curve and expressed as pg/ml.

2.5. Statistical analysis

Statistical examinations of the two vehicle exposures (saline and olive oil) found no significant differences in vehicle-related effects on plasma OT/VP expression (data not shown). Therefore, the decision was made to combine the vehicle groups to form a collective "control/vehicle" exposure group. All statistical analyses and graphical representations were done using GraphPad Prism® 7 (GraphPad Prism® Software, San Diego, USA, www.graphpad.com). The Shapiro-Wilk test was applied to assess for normality of all data with $p < 0.05$ indicating a violation of the assumption of normality. Consequently, all data were analyzed by non-parametric Kruskal-Wallis analysis-of-variance (ANOVA) followed by Dunn's multiple comparisons post-hoc analysis to identify basic main effects or interactions. All data are presented as mean ± standard error of the mean (SEM), where $p \leq 0.05$ is deemed statistically significant. When no significant interactions or basic main effects were noted following ANOVA, or when no statistically significant differences were demonstrated following post-hoc analyses, or when effect-magnitude (relational strength) quantification between variables (where a potential effect is identified using ANOVA) was crucial to data conveyance and interpretation (suggesting significant practical and translational implications), Cohen's *d*-value calculation was performed to calculate effect-sizes between exposures (Lakens, 2013). The aforementioned effect-sizes, as for *non-parametric* analyses, are categorized as follows: medium ($0.3 \geq d \leq 0.5$), large ($0.5 \geq d \leq 0.8$), and very large ($d \geq 0.8$) effect-size. In this study, all datasets demonstrating statistically significant differences ($p \leq 0.05$) were also found to have large/very large effect-sizes as categorized above (data not shown). Therefore, to avoid reader confusion as well as data-overload only significant Cohen's *d*-values (large/very large effect-sizes), where statistical significance was missed, are mentioned and described in the text for the data illustrated in Figs. 1 and 2.

3. Results

3.1. Plasma oxytocin (OT)

All datasets failed to meet the assumption of normality (Shapiro-Wilk; $p < 0.05$), therefore a non-parametric Kruskal-Wallis ANOVA was applied followed by Dunn's post-hoc analysis. A significant main effect of drug exposure on plasma OT concentrations was demonstrated (χ^2 [4] = 47.7, $p < 0.0001$). Dunn's post-hoc analysis revealed a significant reduction in plasma OT levels for EFV ($p < 0.0001$), Δ^9-THC

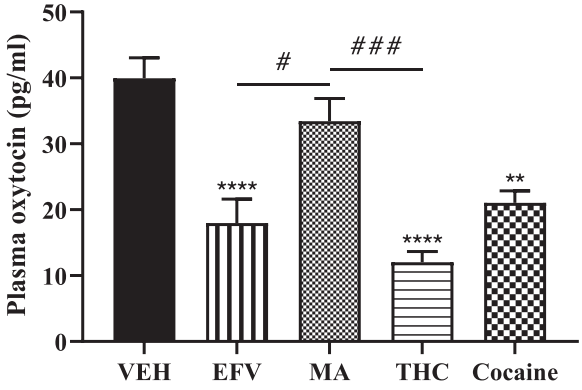

Fig. 1. Effect of the different sub-chronic drug exposures on plasma oxytocin (OT) concentrations (pg/ml) in male Sprague Dawley rats compared to control exposure (n = 16 per exposure group). $^{**}p \leq 0.01$ and $^{****}p \leq 0.0001$ vs. vehicle control. $^{\#}p \leq 0.05$ and $^{\#\#\#}p \leq 0.001$ vs. indicated drug exposure. VEH: vehicle control, EFV: efavirenz (5 mg/kg), Δ^9-THC: Δ^9-tetrahydrocannabinol (0.75 mg/kg), MA: methamphetamine (1 mg/kg), cocaine (20 mg/kg). Cohen's *d*-values not indicated on the figure; please refer to the text (Section 3.1 Plasma oxytocin (OT)).

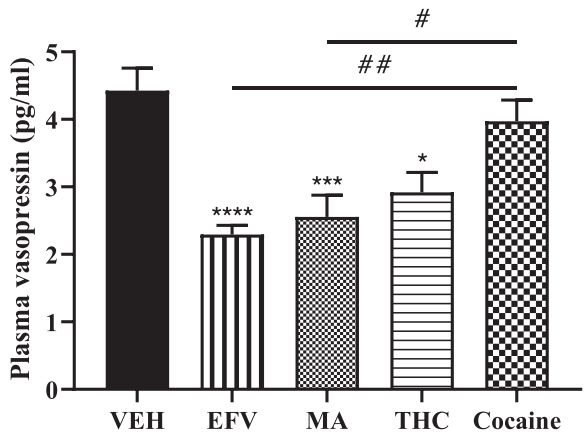

Fig. 2. Effect of the different sub-chronic drug exposures on plasma vasopressin (VP) concentrations (pg/ml) in male Sprague Dawley rats compared to control exposure (n = 16 per exposure group). $^{*}p \leq 0.05$, $^{***}p \leq 0.001$ and $^{****}p \leq 0.0001$ vs. vehicle control. $^{\#}p \leq 0.05$ and $^{\#\#}p \leq 0.01$ vs. indicated drug exposure. VEH: vehicle control, EFV: efavirenz (5 mg/kg), Δ^9-THC: Δ^9-tetrahydrocannabinol (0.75 mg/kg), MA: methamphetamine (1 mg/kg), cocaine (20 mg/kg). Cohen's *d*-values not indicated on the figure; please refer to the text (Section 3.2 Plasma vasopressin (VP)).

($p < 0.0001$) as well as for cocaine-exposed animals ($p = 0.0023$) compared to vehicle control exposure (Fig. 1). EFV ($p = 0.0132$) and Δ^9-THC ($p = 0.0002$) further showed greater significance in reducing plasma OT levels compared to MA, the latter having no effect on OT concentrations compared to vehicle exposed animals ($p > 0.05$). A large effect-size ($d = 0.57$) and very large effect-size ($d = 1.31$) was observed between animals exposed to Δ^9-THC vs. EFV and Δ^9-THC vs. cocaine, respectively, suggesting Δ^9-THC as having the greater effect in reducing plasma OT levels between the different DOA exposures.

3.2. Plasma vasopressin (VP)

The assumption of normality was not met by the datasets representing the effects of the various exposures on plasma VP concentrations (Shapiro-Wilk; $p < 0.05$). A Kruskal-Wallis ANOVA revealed a significant main effect of drug exposure on plasma VP levels for the different exposure groups (Fig. 2: (χ^2 [4] = 33.3, $p < 0.0001$), with Dunn's post-hoc test indicating that EFV ($p < 0.0001$), MA ($p = 0.0001$) and Δ^9-THC ($p = 0.0141$) significantly reduced plasma VP levels compared to the vehicle exposed animals. Furthermore, Dunn's post-hoc analysis also demonstrates that EFV ($p = 0.0046$) and MA ($p = 0.0113$) significantly reduced plasma VP when compared to cocaine-exposed animals. The latter exposure group did not significantly affect plasma VP levels ($p > 0.05$). A large effect-size ($d = 0.73$) was evident for EFV vs. Δ^9-THC suggesting EFV to have a greater effect in reducing plasma VP concentrations between the two drugs. A very large effect-size was also evident between Δ^9-THC and cocaine ($d = 0.87$).

4. Discussion

This study shows for the first time that sub-chronic EFV exposure reduces plasma levels of both OT and VP, in a manner comparable to that of known DOA (viz. MA, Δ^9-THC and cocaine). Reports of EFV abuse in combination with other illicit substances, as the drug combination *Nyaope/Whoonga* (Grelotti et al., 2014; Morris, 2014; Rough et al., 2014), has sparked great interest into understanding the various mechanisms by which EFV exerts its neuropsychological and bio-behavioral effects and to what extent these effects correlate with those induced by other DOA (Dalwadi et al., 2016; Gatch et al., 2013). Of note, although sex-related differences are not down-played in our study (see Section *2.1 Animals*), the illicit use of EFV in combination with other known DOA (viz. as *Nyaope*) is reported to occur more frequently under men than women (Dintwe, 2017). Nevertheless, there is potential for gender-associated variations in response to EFV, as with other DOA (Becker and Koob, 2016), Indeed, sex-based variations in OT and VP expression are well-described (as reviewed by Wilkinson and Brown, 2015).

Importantly, the observed neuropeptide changes are resultant of a sub-chronic intermittent dosing protocol associated with the CPP paradigm, a drug-addiction paradigm, as previously performed in our laboratories (Möller et al., 2018). This protocol takes into consideration the alternation between vehicle and drug exposure, emphasizing a typical binging pattern associated with DOA (Sun et al., 2018). Indeed, such a binging pattern with sub-chronic EFV at the dose used here has previously demonstrated addictive-like behavior in rats (Möller et al., 2018). Likewise, the applied dosages of MA, Δ^9-THC and cocaine also demonstrate addictive-like behavior in rodents using the same behavioral paradigm (Braida et al., 2004; Nygard et al., 2015; Xu et al., 2016). Though the animals in our study were not subjected to behavioral analyses, to our knowledge there is no literature evidence to suggest that subjecting a rodent model to the CPP behavioral assay may lead to significant changes in central and/or peripheral OT/VP levels that may result in confounding bio-/neurochemical outcomes. Additionally, the outcome of this study was not aimed at determining whether behavioral changes resulted from the observed drug-induced neuropeptide changes.

The results with MA, Δ^9-THC and cocaine appear to be in line with that of previous findings in both rodent plasma and various brain regions, such as the hypothalamus, nucleus accumbens (NAc), amygdala and hippocampus (Georgiou et al., 2016; Johns et al., 1998; Sarnyai et al., 1992a, 1992b; also see Buisman-Pijlman et al., 2014; Lee et al., 2016 for review). Though peripheral neuropeptide expression cannot directly account for drug-related changes in the HNS, and vice versa (as reviewed by Leng and Ludwig, 2016; Neumann and Landgraf, 2012), plasma neuropeptide measurements (and accompanying psychobehavioral changes) may yet be translatable to humans (Gouin et al., 2012; Sarnyai, 1999), as evinced from both clinical (Light et al., 2004) and preclinical (McMurray et al., 2008) findings (also see Williams et al., 2012). While not definitive, the current study suggests a probable role for OT and VP in EFV-induced NPAEs and its alleged *addictive* effects (as reviewed by Dalwadi et al., 2018). Undoubtedly, the HNS and its associated neuropeptide hormones play a significant role in neuropsychological conditions such as addiction, affective and cognitive disorders (see Godino and Renard, 2018; Neumann and Landgraf, 2012 for review).

Although Δ^9-THC and cocaine showed a similar reduction in OT, this was not the case with MA. However, MA exposure significantly reduced plasma VP levels. While sub-chronic cocaine exposure significantly reduced plasma OT levels, it had no noteworthy effect on VP concentrations. Interestingly though, EFV and Δ^9-THC were the only two drugs to significantly reduce *both* OT and VP plasma concentrations. This finding may have particular relevance for their apparent preferred abuse potential in combination, e.g. *Nyaope/Whoonga*. It is nonetheless important to note that EFV shows a larger effect-size decrease in VP levels vs. that of Δ^9-THC, whereas this relationship is reversed for plasma OT. These two drugs are therefore unique in how they alter these two neuropeptides. Thus, prolonged exposure to EFV alters peripheral neuropeptide levels in a manner near parallel to that induced by DOA. Whether such changes reflect alterations in CNS OT/VP release and function requires further study, e.g. OT/VP receptor studies.

Since DOA interfere with multiple neurological systems involving inter alia DA, 5-HT, NE, GABA and Glu (as reviewed by Uhl et al., 2019), which are also systems capable of modulating or being modulated by OT and/or VP (see Bowen and Neumann, 2017; Leong et al., 2018 for review), further study is needed to better understand and interpret the actions of EFV described here. In fact, OT and VP receptors are expressed on cortical GABAergic and glutamatergic neurons, NAc astrocytes, and

GABA and Glu-synthesizing neurons in reward-associated brain regions and so are empowered to modulate reward-driven behaviors (Leong et al., 2018).

Methamphetamine exposure had no effect on plasma OT, yet significantly reduced plasma VP concentrations (Figs. 1 and 2, center panel; respectively). Interestingly, when comparing MA to cocaine (Figs. 1 and 2, far-right panel), it appears the two drugs have opposing effects, with MA only affecting plasma VP and cocaine affecting OT. A potential explanation for the observed outcome involves increased synaptic monoamine availability via inhibition of monoamine oxidase A (MAO-A), general monoamine transporters and vesicular monoamine transporter-2 ($VMAT_2$) (Cruickshank and Dyer, 2009), and indirectly activating monoamine receptors. Long-term MA abuse may prompt HPA-axis dysregulation, further impacting neuropeptide release (Baracz et al., 2016; Hostetler et al., 2016; Zuloaga et al., 2015). Contradictory to our results, however, is research in prairie voles (Hostetler et al., 2016) and other small rodents (Baracz et al., 2016; Holubová et al., 2019; see Lee et al., 2016 for review) showing significantly reduced or increased hypothalamic or plasma OT levels in addition to causing regional brain OTR up-regulation (Baracz et al., 2016; Zanos et al., 2014). These discrepancies in results may stem from the use of rodent models from different developmental phases, different drug doses, or a difference in dosing regimen and route of administration. It may also be that the MA dose applied in our investigation was too low to induce significant plasma OT fluctuations and may, therefore, warrant further exploration. The MA-induced decrease in plasma VP may be a result of either central and/or peripheral mechanisms. Frequent MA abuse leads to modified HPA-axis morphology and functionality, thereby affecting the way in which VP synthesis and release are controlled (Zuloaga et al., 2015), a process that may underlie our observations. Reduced VP-release may also result from the neurotoxic effects associated with chronic MA exposure (Cruickshank and Dyer, 2009; Leong et al., 2018). Finally, cardiovascular dysfunction (e.g. hypertension, cardiomyopathy, cardiac hypertrophy, and arterial disease) following chronic MA exposure (Kaye et al., 2007) may promote a negative feedback response to reduce VP synthesis and release (see Leong et al., 2018 for a review on the various cardiovascular effects associated with MA abuse).

Cocaine significantly decreased plasma OT but did not affect plasma VP (Figs. 1 and 2, far-right panel; respectively), which coincides with previous in vivo research (Light et al., 2004; Sarnyai et al., 1992a, 1992b; also see Williams and Johns, 2014; Sarnyai, 1999 for review), with other studies demonstrating similar findings in rodent brain tissue (Elliott et al., 2001; Johns et al., 1993, 1998, 2010). Sarnyai and colleagues screened for both central and peripheral OT/VP content after repeated cocaine administration (7.5 mg/kg/twice daily; for 4 days, subcutaneously (SC)) in rats and found that the drug caused a decrease in peripheral and central (viz. hypothalamic and hippocampal) OT concentrations. The aforementioned effects, with specific reference to OT, may be related to OTR up-regulation, potentially mediated by reduced systemic OT levels, which may be indicative of a compensatory response (Sarnyai et al., 1992a, 1992b). Pregnant SD dams subjected to chronic cocaine exposure (15 mg/kg/twice daily or intermittently for 20 days) were also found to present with reduced OT levels in the amygdala (Johns et al., 1998). Cocaine is able to interact with a variety of neurological systems (i.e. GABAergic, glutamatergic and endocannabinoid) in addition to prompting increases in synaptic neurotransmitter concentrations, similar to MA (Ciccarone, 2011; Simon and Kreek, 2016; Spronk et al., 2013). By interacting with the ECS (Spronk et al., 2013) and regulating the HPA-axis (Simon and Kreek, 2016), it is enabled to modulate both oxytocinergic and vasopressogenic neurocircuitry (Tasker et al., 2015). While reduced plasma OT concentrations observed here may arise from monoaminergic interactions (Sarnyai et al., 1992b; Wilkinson and Brown, 2015), lower OT levels may also be a result of cocaine-induced neurotoxicity to hypothalamic oxytocinergic neurons culminating in oxytocinergic dysregulation (Neumann and Landgraf, 2012).

Regarding Δ^9-THC, the drug's psychoactive properties are primarily mediated by CB_1 receptors (Kendall and Yudowski, 2017; Murray and Bevins, 2010) and OT/VP release may be subject to modulation by co-localized expression of said receptors in the PVN and SON (Rettori et al., 2010). The hypothalamus displays a low density of CB_1 receptors, although their activation produces a greater signal magnitude (Murray and Bevins, 2010). Furthermore, not unlike other DOA, Δ^9-THC also increases monoamine availability by inhibiting MAO-A (Fišar, 2010). Our findings show that Δ^9-THC significantly reduced peripheral levels of *both* OT and VP (vs. control) while having a larger effect versus other DOA exposures. These results potentially correlate with reduced OT levels in brain reward centers following Δ^9-THC exposure (1.5 mg/kg/day, IP, over 7 days) described by Butovksy and colleagues (2006), although plasma OT (or VP) were not measured. Specifically, Δ^9-THC had a greater effect on OT vs. EFV, MA and cocaine and on VP vs. EFV and cocaine (Figs. 1 and 2, second panel from the right). These actions of Δ^9-THC may stem from its activity within the ECS and the latter's interaction with the HNS, although the precise mechanisms are not clear (see Busquets-Garcia et al., 2018; Kendall and Yudowski, 2017). Monoamine alterations brought on by Δ^9-THC, as noted earlier, in addition to its actions on the ECS and HNS, may also be responsible for changes in OT and VP secretion (as reviewed by Wilkinson and Brown, 2015).

That EFV significantly suppressed plasma levels of both OT and VP (Figs. 1 and 2, second panel from the left) is compelling, although the underlying mechanisms require further study. Interestingly though, Δ^9-THC had a greater inhibitory effect on OT whereas EFV has a larger effect on VP. While speculative, the observed peripheral changes in OT and VP may have resulted from EFV-induced modifications to oxytocinergic and vasopressogenic neurocircuitry, probably as a consequence of the drug's ability to cross into the CNS (Decloedt et al., 2015). However, it needs to be borne in mind that peripheral OT/VP changes and their association with OT/VP circuitry in the brain are subject to inter-modulatory actions of a number of systems, which require study. Moreover, EFV modulates numerous neurological systems (i.e. serotonergic, muscarinic and GABAergic) that have been suggested to underlie EFV-induced NPAEs (Dalwadi et al., 2016; see Dalwadi et al., 2018 for review; Gatch et al., 2013; Huang et al., 2017). EFV, at the dose used in this study, also increases striatal DA levels and reward-driven behavior (Möller et al., 2018), thereby implicating dopaminergic reward neurocircuitry in this action. EFV also modulates DAT, serotonin transporter (SERT), $VMAT_2$ and MAO-A activity; similar to other DOA (as reviewed by Dalwadi et al., 2018). Taken together, EFV exhibits a similar neuropsychopharmacological profile to that of MA, Δ^9-THC and cocaine and may, as a result, exert comparable or even greater effects on the HNS and its associated neuropeptides.

Limitations and future study: There are reports that *Nyaope/Whoonga* abuse occurs more frequently under men than women (Dintwe, 2017). The examination of sexual (male vs. female SD rat) dimorphisms (if any) in terms of drug-responding and drug-induced alterations in OT/VP release or levels, as it relates to the different investigated drug exposures, would therefore be beneficial. The assessment of neuropeptide levels from both central and peripheral biological samples would assist in demonstrating comparable compartmental effects. Since EFV is purportedly used in conjunction with other psychoactive substances to enhance euphoretic sensations, it would be valuable to assess the effects of EFV when used in combination with MA/Δ^9-THC and/or cocaine on central/peripheral neuropeptide expression. Dose-response studies on EFV-associated effects on HNS function would also confirm the findings presented here. Finally, combining EFV-induced behavioral investigations with targeted biomarker analysis would provide a clearer interpretation of the underlying biological mechanisms involved in the observed psychoactive properties of EFV.

5. Conclusions

Prolonged EFV exposure significantly reduces plasma OT and VP concentrations in a comparable way to Δ^9-THC, albeit having a greater effect on VP than OT, and has a more comparable profile to Δ^9-THC than MA and cocaine. This adds a new dimension to EFV's potential to precipitate neuropsychological sequelae and substance abuse (Dalwadi et al., 2018). EFV-induced HNS dysfunction involving oxytocinergic and vasopressogenic neurocircuitry may lay the foundation for various psychological/psycho-endocrine maladies, including depression- and anxiety-related disorders, learning and memory deficiencies as well as social impairments (see Neumann and Landgraf, 2012; Stoop, 2012 for review). Finally, this action may promote abuse of EFV itself or that of other DOA. Our findings are of significant importance in providing new insights into potential mechanisms underlying the illicit application of EFV for its alleged euphoric (Gatch et al., 2013) and/or rewarding effects (Möller et al., 2018; also reviewed by Dalwadi et al., 2018).

Ethics approval and consent to participate

All experimental procedures involving Sprague-Dawley rats were conducted according to the Animal Research: Reporting *In Vivo* Experiments (ARRIVE) guidelines with the approval (ethics approval number: NWU-00291-17-A5) of the North-West University (NWU) animal research ethics committee, AnimCare (NHREC reg. number AREC-130913-015), Potchefstroom, South Africa.

Consent for publication

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CRediT authorship contribution statement

Mandi Le Roux: Research and investigation processes (viz. all exposure-related procedures and biological investigations); Data acquisition, analysis and visualization (i.e. processing, graphic illustration and interpretation of data), Resource procurement (i.e. materials, reagents, and laboratory samples); Writing of the original draft (i.e. further editing and review of drafts). **Marisa Möller:** Study design/conceptualization; Data analysis, elucidation and visualization; Study supervision; Project administration; Resource support; Funding acquisition; Writing - review & editing. **Brian H. Harvey:** Data elucidation and visualization; Study supervision (viz. co-supervisor); Writing - review & editing. The final version of the manuscript was read, revised and approved by all the author(s) prior to its submission.

Declarations of interest

The author(s) have no competing interests to declare with regards to the research, manuscript authorship, and/or publication of this article. Excluding income received from the chief employer and financial research support granted to M. Möller from the abovementioned organizations, the author(s) declare that no additional funding or compensation has been received from an individual or corporate entity in addition to there being no personal financial holdings that could be perceived as constituting a personal conflict of interest.

Availability of data and materials

All data produced and evaluated during this study are obtainable and will be made available from the corresponding author upon reasonable request.

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