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Highlights

- FORNET treatment reduces appetitive aggression and PTSD symptom scores
- *AUTS2* and *NR4A2* methylation are inversely associated with PTSD symptom severity
- *TFAM* methylation is positively associated with appetitive aggression scale scores
- Dopaminergic signalling and synaptic plasticity influence PTSD symptom severity
- Oxidative phosphorylation capacity is implicated in appetitive aggression

DNA methylation and psychotherapy response in trauma-exposed men with appetitive aggression

Khethelo R. Xulu[a]^, Jacqueline S. Womersley[a,b]^, Jessica Sommer[c]; Martina Hinsberger[c]; Thomas Elbert[c], Roland Weierstall[c,d]; Debbie Kaminer[e]; Stefanie Malan-Müller[a]; Soraya Seedat[a,b]; Sian M. J. Hemmings[a,b]*

[a] Department of Psychiatry, Faculty of Medicine & Health Sciences, Stellenbosch University, Cape Town, South Africa; [b] South African Medical Research Council / Stellenbosch University Genomics of Brain Disorders Research Unit, Faculty of Medicine & Health Sciences, Stellenbosch University, Cape Town, South Africa; [c] Department of Psychology, University of Konstanz, Konstanz, Germany; [d] Clinical Psychology & Psychotherapy, Medical School Hamburg, Hamburg, Germany; [e] Department of Psychology, University of Cape Town, Cape Town, South Africa

^ These authors contributed jointly as first authors

Khethelo R. Xulu	xuluk2@ukzn.ac.za
Jacqueline S. Womersley	jsw1@sun.ac.za
Jessica Sommer	jessica.sommer@institut-fuer-ppt.de
Martina Hinsberger	martina.hinsberger@gmail.com
Thomas Elbert	Thomas.Elbert@uni-konstanz.de
Roland Weierstall	roland.weierstall@medicalschool-hamburg.de
Debbie Kaminer	Debbie.Kaminer@uct.ac.za
Stefanie Malan-Müller	smalanmul@gmail.com
Soraya Seedat	sseedat@sun.ac.za
Sian M. J. Hemmings	smjh@sun.ac.za

*Corresponding author: Sian Hemmings

Department of Psychiatry

Stellenbosch University, Faculty of Medicine & Health Sciences

P.O. Box 241

Cape Town

8000

South Africa

smjh@sun.ac.za

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Abstract

Exposure to violence can lead to appetitive aggression (AA), the positive feeling and fascination associated with violence, and posttraumatic stress disorder (PTSD), characterised by hyperarousal, reexperience and feelings of ongoing threat. Psychotherapeutic interventions may act via DNA methylation, an environmentally sensitive epigenetic mechanism that can influence gene expression. We investigated epigenetic signatures of psychotherapy for PTSD and AA symptoms in South African men with chronic trauma exposure. Participants were assigned to one of three groups: narrative exposure therapy for forensic offender rehabilitation (FORNET), cognitive behavioural therapy or waiting list control (n = 9-10/group). Participants provided saliva and completed the Appetitive Aggression Scale and PTSD Symptom Severity Index at baseline, 8-month and 16-month follow-up. The relationship, over time, between methylation in 22 gene promoter region sites, symptom scores, and treatment was assessed using linear mixed models. Compared to baseline, PTSD and AA symptom severity were significantly reduced at 8 and 16 months, respectively, in the FORNET group. Increased methylation of genes implicated in dopaminergic neurotransmission (*NR4A2*) and synaptic plasticity (*AUTS2*) was associated with reduced PTSD symptom severity in participants receiving FORNET. Analyses across participants revealed a proportional relationship between AA and methylation of *TFAM*, a gene involved in mitochondrial biosynthesis.

Keywords

DNA methylation; epigenetics; appetitive aggression; PTSD; violence; psychotherapy

1. Introduction

The understanding of violence, i.e. actions that use threatened or actual force with the intent to cause harm, has evolved from a legal, judicial and policing focus to one that recognises its fundamental impacts on the health of individuals and communities (Bowman et al., 2015; Hoeffler, 2017). Violence was declared a worldwide leading public health problem by the World Health Organisation in 1996 (World Health Assembly, 49, 1996).

Cumulative exposure to traumatic stressors has been linked to the development of a range of adverse effects on mental health and psychosocial functioning (Ainamani et al., 2017; Köbach et al., 2015). Trauma experience lays down strong associative memories in fear networks such that trauma-associated cues elicit a fear response (Hecker et al., 2015). With increasing trauma, these fear responses are generalised beyond specific trauma cues, generating a chronic sense of threat

and vigilance (Hecker et al., 2015), as is characteristic of posttraumatic stress disorder (PTSD) (American Psychiatric Association, 2013). Continued exposure to violence can also lead to aggression. In a process of decontextualization, cues previously associated with trauma may develop a fascinating, exciting, rewarding or arousing quality, engaging a "hunting network" (Elbert et al., 2018, 2017, 2010; Hecker et al., 2015). Though seemingly counterintuitive, this goal-directed and predatory attraction to violent behaviour, termed appetitive aggression (AA), may be a protective mechanism with the reward experienced in perpetrating violence attenuating the harm associated with PTSD symptoms (Hecker et al., 2013; Hinsberger et al., 2016; Weierstall et al., 2012, 2011). However, the relationship between AA and PTSD is more complex than this simple dichotomy. The linear relationship between the number of violent acts perpetrated and AA can persist over years, contributing to a cycle of violence (Hermenau et al., 2013; Köbach et al., 2015; Mueller-Bamouh et al., 2016; Nandi et al., 2015). Furthermore, an AA-associated increase in the probability of engaging in violence can increase the risk of victimisation (Weierstall et al., 2012). Thus, individuals living in environments characterised by chronic violence can develop a parallel victim-offender status, perpetuating environments that foster aggression and further trauma (Hinsberger et al., 2016).

The burden of violence, and corresponding psychiatric and behavioural disorders, is particularly felt in low- and middle-income countries (Matzopoulos et al., 2008). Within South Africa, the complex mesh of growing economic and structural inequality, poverty, unemployment, early adversity, historical disenfranchisement and repressive political regimes, weaknesses in law enforcement, access to firearms, alcohol, normalisation of violence and patriarchal masculinity that values toughness provide fertile ground for the high levels of interpersonal, community and structural violence that exist (Bowman et al., 2015; Seedat et al., 2009). Community violence is especially

Abbreviations:

AA = appetitive aggression; CBT = cognitive behavioural therapy; CECV = Childhood Exposure to Community Violence; FORNET = Narrative Exposure Therapy for Forensic Offender Rehabilitation; PCR = polymerase chain reaction; PSS-I = PTSD Symptom Scale - Interview; PTSD = posttraumatic stress disorder; WL = waitlisted; SD = standard deviation

problematic in townships i.e. areas that were previously designated for Black South Africans under the Apartheid system of government, where residents are repeatedly exposed to high levels of neighbourhood, gang, school and police violence, with potent effects on mental health (Martin et al., 2013; Shields et al., 2008; Stansfeld et al., 2017)

Trauma-informed psychotherapeutic interventions that address symptoms of trauma and seek to rebuild social relationships and allow reintegration into communities are key to fostering positive cycles of community improvement and violence de-escalation. The aim of Narrative Exposure Therapy for Forensic Offender Rehabilitation (FORNET) is to ameliorate the harmful effects of chronic trauma exposure and reduce the pleasure and fascination associated with aggression and violence (Hecker et al., 2015; Hinsberger et al., 2017). By verbally sharing experiences of witnessed and experienced trauma, disordered thinking can be evaluated and reframed, memories reorganised and the impact of trauma recognised with the end goal of reducing AA, and more specifically, the

likelihood of acting on aggressive urges (Hecker et al., 2015; Hinsberger et al., 2017). Thinking for a Change (TFAC) is a cognitive behavioural therapy (CBT) intervention that targets habits, thinking and attitudes that support aggression and offending, and aims to improve social skills and problem-solving such that altered cognitions can produce downstream changes on behaviour (Hinsberger et al., 2017). Though FORNET and CBT differ in their approaches, both show promise in addressing the consequences of trauma, with FORNET associated with reductions in PTSD symptom severity and the likelihood of perpetrating violent acts (Crombach and Elbert, 2015; Köbach et al., 2017), and CBT associated with improved problem-solving skills and reduced recidivism (Golden et al., 2006; Lowenkamp et al., 2009).

Though many studies have examined the biological mechanisms underlying risk and resilience in PTSD and aggressive behaviour (Bartholow, 2018; Hammamieh et al., 2017; Provencal et al., 2015; Sharma and Ressler, 2019; Waltes et al., 2016), researchers have only recently begun to investigate the biological mechanisms underlying sensitivity to therapeutic interventions (Kumsta, 2019; Schiele et al., 2020). Epigenetic modifications, i.e. stable but potentially reversible structural alterations to genes that influence gene expression, offer one means by which experiences can become biologically embedded (Schiele et al., 2020). The most widely studied of these mechanisms is DNA methylation, which refers to the covalent binding of a methyl group with a cytosine residue in CpG dinucleotide sequences (Moore et al., 2013). Environmentally induced changes in methylation profile allow modification of gene expression in response to subsequent environmental triggers, enabling plastic brain responses that are contingent on previous experience (Abdolmaleky et al., 2004; Schiele et al., 2020).

The current study seeks to investigate alterations in DNA methylation in response to psychotherapy in men with high AA and PTSD symptoms. The study was nested within a larger investigation of the role of chronic trauma and violence exposure in AA and PTSD in young men in South Africa (Hinsberger et al., 2016). The parent study examined whether attraction to violence in the context of chronic trauma exposure is associated with PTSD severity and perpetration of violence in 290 men recruited from two low-income community settings in Cape Town, South Africa (Hinsberger et al., 2016). The results indicated that AA was positively associated with witnessed and experienced trauma, and in turn predicted both higher levels of PTSD symptoms and perpetrated violence (Hinsberger et al., 2016; Sommer et al., 2017a). PTSD symptoms were further correlated with experienced traumatic events (Hinsberger et al., 2016). The value of FORNET and CBT psychotherapeutic interventions on PTSD and AA symptoms, as well as perpetrated violence, were investigated in a longitudinal study that specifically targeted young males who both experienced and perpetrated violence i.e. the group most likely to be seriously impacted by violent crime in urban areas (Hinsberger et al., 2017). Data obtained from the 39 participants [FORNET $n = 15$, CBT $n = 11$ and waitlisted (WL) controls ($n = 13$] indicated that FORNET was associated with significantly reduced PTSD symptom severity at both eight- and sixteen-month follow-up (Hinsberger et al., 2019, 2017). Though FORNET was also associated with reduced AA at sixteen months, this did not translate into a reduction in perpetrated violence (Hinsberger et al., 2019). The present study examines longitudinal DNA methylation and symptom profiles in 29 of the participants enrolled in the

intervention study. We hypothesised that psychotherapy-associated changes in AA and PTSD symptoms would be mirrored by altered methylation signatures in genes targeted for their role in psychiatric disorders.

2. Methods

2.1. Study design and patient population

Participants were recruited in 2013 and 2014 using the assistance of a local organisation, REALISTIC (Rebuilding and Life Skill Training Centre). This programme operates in the Gugulethu and Khayelitsha townships of Cape Town, South Africa and seeks to reintegrate former juvenile off enders into society, family and work (Hinsberger et al., 2016). Participants with acute psychosis were excluded. To be included participants had to have a minimum score of eight on the PTSD Symptom Scale - Interview (PSS-I) and a minimum of nine points on the Appetitive Aggression Scale (AAS). Based on these criteria, 29 participants were randomly selected to provide saliva samples for DNA methylation analyses.

Ethical clearance was obtained from each of the human research ethics review boards of Stellenbosch University, the University of Cape Town and the University of Konstanz. All participants provided written informed consent, and where participants were below the age of majority, parental/guardian consent with participant assent was obtained.

2.2. Assessment instruments

Assessments were conducted using structured interviews with trained isiXhosa interpreters when necessary. All study instruments were translated and back translated to provide bilingual (English/isiXhosa) options. All interviewers were blind to the treatment condition of participants.

PTSD symptoms were assessed using the PTSD Symptom Scale - Interview (PSS-I), which includes seventeen questions designed to measure the frequency and intensity of PTSD symptoms experienced in the preceding two weeks using a four-point Likert scale, producing a possible range of scores from zero to 51 points (Foa and Tolin, 2000). We assessed participant attraction to and desire to commit violent acts using the AAS, a semi-structured interview in which participants are asked to agree or disagree with fifteen statements using a five-point Likert scale. The sum score (with a possible range of zero – 60) is proportional to AA (Weierstall and Elbert, 2011). Trauma exposure was assessed using the Childhood Exposure to Community Violence Checklist (CECV), a 33-item self-report checklist that assesses the frequency of witnessing, hearing about and experiencing violence in childhood and adolescence using a five-point Likert scale (Amaya-Jackson, 1998). In order to better reflect the nature of violence experienced in South African townships, a locally adapted 36-item version including assessment of physical assault, armed robbery and sexual abuse was used (Sommer et al., 2017b), yielding a possible range of scores of zero - 36. Analysis of the data obtained from the larger intervention study with 39 participants indicated that these measures had excellent

internal consistency [PSS-I Cronbach's alpha = 0.88; AAS Cronbach's alpha = 0.86 and CECV Kudar-Richardson's alpha = 0.86] (Hinsberger et al., 2017).

2.3. Psychotherapeutic interventions

The details of the psychotherapy effects on mental symptoms have been previously reported (Hinsberger et al., 2017). Briefly, participants were randomly allocated to three treatment groups which were matched on PSS-I, AAS and CECV scores (Hinsberger et al., 2017).

The interventions were conducted at several three-week camps, each hosting twelve to fourteen participants, in order to allow psychotherapy to be delivered without concerns around safety, drugs, shelter, and nutrition (Hinsberger et al., 2017). Therapy sessions were conducted by four German and five South African mental health experts with training in FORNET or CBT and took place in separate rooms to ensure privacy and confidentiality (Hinsberger et al., 2017). The FORNET intervention consisted of eight two-hour sessions covering psychoeducation around PTSD; collection of biographical information; selection, recounting and sensory re-experiencing of the index trauma; reanalysis of the timeline of trauma exposure, and finally future outlooks and expressions of hope (Hinsberger et al., 2017). The CBT intervention consisted of seven two-hour sessions and included information on the relationship between cognition and behaviour; evaluation and reframing of thinking patterns; social skills and problem-solving and finally evaluation (Hinsberger et al., 2017). Participants in the control group attended the camps but were not involved in any psychotherapy sessions.

2.4. DNA methylation analyses

DNA methylation analyses were performed for 29 participants (*n* = 10 FORNET, *n* = 10 CBT, *n* = 9 WL). Saliva samples were collected under the supervision of a clinical researcher at three time points (baseline, 8-month and 16-month follow-up) using Oragene DNA self-collection kits (DNA Genotek, Ontario, Canada) as per the kit pamphlet. Saliva samples were stored at room temperature until genomic DNA extraction using Prep-It L2P reagent (DNA Genotek). Following confirmation of DNA quantity and quality via UV spectrophotometry, DNA methylation was investigated using the EpiTect Methyl II Signature PCR Array (22) for Human Mental Disorders (Qiagen, Hilden, Germany). This disease-focussed gene panel uses primers designed to simultaneously assess methylation status in the promoter regions of 22 genes, targeted for their role in neurological and psychiatric disorders, with high specificity and amplification efficacy (Table 1). Following DNA cleavage using methylation-sensitive and methylation-dependent restriction enzymes, real-time polymerase chain reaction was performed using primers designed to flank the targeted promoter regions. The qPCR results, in turn, were used to generate percentages of methylated and unmethylated DNA, thereby allowing comparison of relative methylation across samples and time points.

The PCR Array was conducted according to manufacturer's instructions. Briefly, 0.5 µg of genomic DNA in restriction digestion reaction mixture was divided across four reaction conditions: 1) a single

restriction enzyme digestion with a methylation-sensitive enzyme; 2) a single restriction enzyme digestion with a methylation-dependent enzyme; 3) a double enzyme digestion with both methylation-sensitive and methylation-dependent enzymes; and 4) a control mock reaction without enzymes. Following overnight incubation at 37 °C, digestion was terminated by heating the samples to 65 °C for 20 min. Samples were subsequently randomly distributed across 384-well reaction plates, each of which included methylation-sensitive and methylation-dependent enzyme controls. PCR reactions using RT^2 SYBR Green Fluor qPCR Mastermix were performed on the ABI Prism 7900HT platform (Applied Biosystems, Foster City, CA). The EpiTect Methyl II PCR Array spreadsheet was populated with raw ΔC_T values for automatic calculation of (un)methylated DNA fractions. This was used to calculate DNA methylation percentages for each gene at each time point.

2.5. Statistical analyses

Methylation data were not normally distributed and were thus natural log-transformed for further analysis. Differences in clinical and demographic measures according to treatment group were assessed using analysis of variance. The longitudinal course of AA and PTSD symptom profiles according to treatment group have been previously assessed and reported for the 39 participants that completed the intervention study (Hinsberger et al., 2019, 2017). These analyses were repeated as this investigation uses data from only a subset of these participants (n = 29). Longitudinal change in symptom scores were assessed within treatment groups using time as a fixed factor and participant as a random factor. Cohen's *d* was calculated to determine the effect size of significant differences. To determine whether changes in percentage methylation mapped to the identified significant differences in symptom scores, we applied linear mixed models with methylation as an outcome variable, time and standardised AAS or PSS-I scores as fixed effects, and participant as a random effect. To investigate the epigenetic profile of FORNET intervention, we generated linear mixed models comparing FORNET and WL groups. These models used methylation as an outcome variable, participants as a random effect, and treatment group, time and standardised AAS or PSS-I scores as fixed effects. Analyses that indicated significant group x time x symptom score interactions were further investigated by calculating Pearson's product moment correlations between methylation and symptom scores within groups and at each timepoint. We determined the significance of the difference between correlation coefficients using pairwise comparison of Fisher's z-transformed correlations. The Cohen's *q* measure of effect size was used to interpret the difference between correlation values. Finally, linear mixed models were also used to assess the relationship between methylation profile and symptom scores in the longitudinal dataset. Methylation was designated as the outcome value, symptom scores were included as a fixed effect whilst participant was included as a random effect to control for repeated measurements within subjects. The degrees of freedom for mixed models were estimated using Satterthwaite approximation and raw regression coefficients are reported. An alpha value of 0.05 was used to determine significance and all post hoc tests employed Bonferroni-Holm correction to control for multiple testing. Analyses were performed using the R statistical language, with *lme4* and *lmertest* packages (Bates et al., 2020; Kuznetsova et al., 2020; R Core Team, 2020). Graphs were generated using GraphPad Prism 5 (GraphPad Software, San Diego, CA).

3. Results

3.1. Clinical and demographic information

All participants self-identified as Black South African males with isiXhosa as their first language. Participants had an average age of 23 years (age range of 18 – 40 years) and reported completing an average of 10.07 years of education (range 1– 14 years). No significant differences in age, CECV, AAS or PSS-I scores were found between groups at baseline. Analysis within treatment groups indicated that, compared to baseline, PSS-I and AAS scores were significantly lower at eight (β = -9.53, t = -2.16, p = 0.043) and sixteen (β = -7.70, t = -2.25, p = 0.037) months respectively in participants receiving FORNET (Table 2 and Figure 1). The calculated Cohen's d values indicated a large effect size for the reduction of PSS-I scores from baseline to eight months (d = 0.91) and a small effect size for the decrease in AAS scores between baseline and sixteen months (d = 0.44). Symptom scores did not change in either CBT or WL groups.

3.2. FORNET treatment response and DNA methylation

We assessed whether the identified treatment effects of FORNET were reflected in changes in DNA methylation. The analyses yielded no significant relationships between the reduction in AAS scores and methylation levels between baseline and 16-month follow-up. However, we found two significant time x PSS-I score effects. The reduction in PSS-I scores between baseline and 8 months was significantly associated with increases in the methylation of both autism susceptibility candidate 2 (*AUTS2)* (β = 0.34, t = 2.47, p = 0.023) and nuclear receptor subfamily 4, group A, member 2 (*NR4A2)* (β = 0.13, t = 2.12, p = 0.049) (Figure 2). Cohen's q values indicated large effect sizes for both findings (*AUTS2* q = 1.23 and *NR4A2* q = 1.00).

Linear mixed models including data from FORNET and WL groups revealed significant time x group x AAS score interaction effects on the methylation status of the islet 2 LIM homeobox gene (β = 2.59, t = 2.35, p = 0.024). Time x group x PSS-I score effect analyses revealed significant effects on the methylation level of *AUTS2* (β = -5.01, t = -2.14, p = 0.037) and the gene encoding arginine vasopressin (β = 3.39, t = 2.22, p = 0.031) at the eight-month timepoint. Significant 16-month x group x PSS-I score effects were found for *AUTS2* (β = -5.62, t = -2.69, p = 0.010), LIM homeobox 5 (β = -5.18, t = -2.92, p = 0.028), and *NR4A2* (β = -3.41, t = -2.43, p = 0.019). Of these findings, only differences in the relationship between *AUTS2* methylation and PSS-I scores at 16 months survived post hoc testing with multiple-testing correction (adjusted p = 0.028) (Figure 2), with a Cohen's q value of 1.58 indicating a large effect size.

3.3. DNA methylation and symptom scores

Cross-sectional analyses using baseline data found no relationship between AAS scores and methylation status for any of the targeted genes. Longitudinal analyses indicated a positive association between methylation of the mitochondrial transcription factor A (*TFAM*) gene and AAS scores (β = 0.06, t = 2.28, p = 0.026) (Figure 3). The Pearson's correlation r value of 0.25 indicates a small effect size.

4. Discussion

This study examined whether DNA methylation, an environmentally-sensitive epigenetic modification which can influence gene expression, is associated with change in AA and PTSD symptoms following psychotherapeutic intervention in a population of offenders resident in areas with high endemic levels of community and gang violence. We further investigated whether changes in AAS and PSS-I scores in participants who received FORNET correlated with methylation status.

The results of our longitudinal symptom change assessment in our study cohort (n = 29) were largely in keeping with those of the parent intervention study (n = 39) (Hinsberger et al., 2019, 2017). Both analyses found that participants receiving FORNET showed a significant reduction in PSS-I scores at the eight-month follow-up timepoint, as well as a significant reduction in AAS scores at sixteen months. However, the parent study's finding of a significant reduction in PTSD symptom severity in FORNET participants at sixteen months was not repeated in our subset analysis.

Our results showed an inverse relationship between *AUTS2* methylation and PSS-I scores in participants receiving FORNET. As all targeted CpG sites in the EpiTect panel are located in the promoter region, this suggests that AUTS2 activity is positively associated with PTSD symptoms. The role of AUTS2 in neurodevelopment has been widely investigated (Monderer-Rothkoff et al., 2019). Within the nucleus, binding of AUTS2 changes polycomb complex I from a transcriptional repressor into a transcriptional activator thereby regulating neuronal gene expression, whilst cytoskeletal AUTS2 is involved in neuronal migration and neurite extension (Gao et al., 2014). AUTS2 has also been implicated in behavioural processes beyond neurodevelopment with substance use studies finding evidence for genetic variation and AUTS2 expression in drug-related reward and responding (Engmann et al., 2017; Schumann et al., 2011). A recent study found that conditional ablation of *AUTS2* expression in the post-weaning period in mice, i.e. after establishment of brain structure, dysregulated synaptogenesis and was associated with increased excitatory synapse numbers in mature neurons (Hori et al., 2019). This suggests that AUTS2 plays an important role in synaptic physiology in the mature brain, and thus methylation-derived differences in AUTS2 activity could influence synaptic plasticity and homeostasis, including how the brain responds to trauma (Heavner and Smith, 2020).

Increased *NR4A2* methylation was associated with a reduction in PSS-I scores in the FORNET group. NR4A2 is a nuclear receptor and transcription factor required for the differentiation and survival of dopaminergic neurons during development (Jakaria et al., 2019). NR4A2 plays an important role in the maintenance of mature dopaminergic neurons by regulating the expression of genes implicated in dopamine synthesis and catabolism, with higher NR4A2 activity associated with increased dopamine levels in mesolimbic and mesocortical pathways (Eells et al., 2002; Jakaria et al., 2019; Rojas et al., 2010). The expression of NR4A2 is stress-responsive, with glucocorticoid stimulation increasing *NR4A2* transcription in the prefrontal cortex, amygdala, hippocampus, hypothalamus,

ventral tegmental area and pituitary gland, areas of the brain that are important for coordinating the acute and chronic responses to stressor exposure (Helbling et al., 2014). NR4A2 modulation of dopaminergic brain areas can influence behaviours directly relevant to PTSD, including learning and memory, and encoding the salience and valence of stimuli (Lee et al., 2016; Stubbendorff and Stevenson, 2020; Verharen et al., 2020).

Previous research suggests that dopamine plays a role in resilience to traumatic stress. Increased expression of genes encoding dopamine receptors and components of the dopamine synthesis pathway, coupled with reduced expression of genes involved in dopamine degradation, was associated with lower acute stress responses to major life events and catastrophes (Azadmarzabadi et al., 2018). Our finding of decreased NR4A2 activity with improvement in PTSD symptoms would seem to contradict this. However, dopaminergic signalling is complex with both positive and aversive stimuli capable of eliciting dopamine neuron activation in a cell population-specific manner (Verharen et al., 2020). Furthermore, the young men participating in our study reside in high community violence areas and the effects of repeated trauma exposure may have been particularly deleterious during adolescence when dopaminergic innervation of the prefrontal cortex increases (Datta and Arnsten, 2019). Stress increases dopaminergic signalling to the prefrontal cortex, where coupled with elevated glucocorticoid levels, it acts to reduce firing of prefrontal cortical neurons projecting to the amygdala, reducing top-down control and heightening emotional responding (Datta and Arnsten, 2019). Thus, our finding of an association between increased *NR4A2* methylation, i.e. lower NR4A2 activity, and lower PSS-I scores could be due to reduced dopaminergic activity in the prefrontal cortex increasing top-down control.

We found a proportional relationship between *TFAM* methylation and AAS scores, i.e. higher self-reported AA was associated with lower TFAM activity. TFAM forms a component of the mitochondrial transcription initiation complex, which is responsible for the maintenance of mitochondrial DNA, and thus the repair and replication of mitochondria (National Center for Biotechnology Information, 2020). As mitochondria supply energy via oxidative phosphorylation, TFAM is, in turn, an important role player in determining the energy available to support neuronal processes (National Center for Biotechnology Information, 2020; Trumpff et al., 2019). This includes neuroendocrine signalling, synaptic plasticity and epigenetic modifications, which coordinate the acute and chronic responses to stress and ultimately give rise to the behavioural manifestations of stress exposure (Hoffmann and Spengler, 2018; Kim et al., 2019; Picard and McEwen, 2018; Rangaraju et al., 2019). The recognition that the ability to respond to stress depends on myriad energy-dependent actions at the molecular level has given rise to the concept of mitochondrial allostatic load, a term which refers to chronic stress-induced structural and functional changes in mitochondria (Picard and McEwen, 2018). In addition to modulating the response to stress, mitochondria are themselves stress-responsive (Eisner et al., 2018; Picard et al., 2018; Picard and McEwen, 2018). Elevated glucocorticoid exposure has been linked to increased circulating mitochondrial DNA, greater mitochondrial damage, a reduction in mitochondrial copy number and a decline in energy production (Bersani et al., 2016; Du et al., 2009; Picard and McEwen, 2018; Trumpff et al., 2019).

Based on these findings, it is likely that an environment characterised by high violence and repeated trauma exposure can increase mitochondrial allostatic load and reduce energy production. Harnessing the advantage of more direct mechanistic testing afforded by animal model studies, researchers uncovered an inverse relationship between oxidative phosphorylation and aggression (Li-Byarlay et al., 2014; Rittschof et al., 2018). In a study that compared threat-elicited gene expression across species, aggressive behaviour in response to territory intrusion or alarm cues was associated with reduced expression of genes involved in oxidative phosphorylation in both honey bee and mouse, suggesting an evolutionarily conserved relationship (Rittschof et al., 2014). Though the mechanisms underlying the oxidative phosphorylation-aggression relationship are unknown, oxidative phosphorylation can impact multiple energy-dependent processes including neurotransmitter synthesis and turnover, neuronal excitability, the rate of action potential firing, and cytoskeletal reorganisation (Chandrasekaran et al., 2015; Kim et al., 2019; Li-Byarlay et al., 2014; Rittschof et al., 2018; Srivastava et al., 2018). Alterations in mitochondrial calcium ion buffering capacity could influence gene expression and altered redox homeostasis can itself serve as a signalling mechanism (Chandrasekaran et al., 2015; Li-Byarlay et al., 2014; Rittschof et al., 2018). As lower TFAM activity reduces mitochondrial repair and replication, this body of research provides support for our finding of an association between increased *TFAM* methylation with increased AAS scores.

Our results support the value of investigating changes in epigenetic signatures as a biological correlate of therapeutic response. In a call for mutually informed research that bridges the disciplines of neuroscience and psychiatry, Kandel (1998) highlighted the value of investigating the role of epigenetics in psychotherapy and treatment. As all behaviour ultimately rests on brain function, genetically and environmentally driven differences in gene expression, and thus neural activity, can produce altered patterns of connectivity and ultimately behaviour. By extension, environmental influences capable of producing long-term changes in behaviour, such as psychotherapy, must produce coordinated changes in gene expression (Kandel, 1998) albeit not necessarily methylation patterns. Recent reviews provide support for such bottom-up epigenetic mechanisms in determining the response to psychotherapy (Kumsta, 2019; Schiele et al., 2020). Studies investigating response to various interventions (prolonged exposure therapy, cognitive behavioural therapy, dialectical behaviour therapy and mindfulness-based stress reduction) in a range of disorders including PTSD, anxiety disorders, bipolar disorder, major depression and panic disorder, have identified altered methylation signatures in *FKBP5, SLC6A4, MAOA, BDNF, APBA3, MCF2,* and *GLUT1,* genes involved in the stress response, neurotransmission, presynaptic signal transduction, neuronal development and energy metabolism (Kahl et al., 2016; Knoblich et al., 2018; Perroud et al., 2013; Roberts et al., 2019, 2015, 2014; Schiele et al., 2018; Thomas et al., 2018; Yehuda et al., 2013; Ziegler et al., 2016).

The findings of this study need to be interpreted in the context of several limitations. Chief amongst these is that recruitment and retention were complicated by challenging living conditions such as drugs, gangs, insecure housing and family responsibilities. The resultant small sample sizes at certain

time points particularly limits our analyses for the CBT group. This hindered our capacity to discern changes in symptom scores due to treatment, as well as how these may be reflected in methylation profiles. Indeed, due to the low number of participants in the CBT group (n = 3 at sixteen months), the analyses to assess the longitudinal relationship between symptom scores and DNA methylation were limited to participants in FORNET and WL groups. Though small samples are not uncommon in the developing field of psychotherapy epigenetics, with six of the thirteen studies included in the review by Schiele et al. (2020) having fewer than 30 cases, it is important to keep in mind that the small sample size reduces the informative value of the reported statistics and the generalisability of our findings. Consequently, our results must be interpreted with a degree of caution and should be taken as an indication of potential, rather than definitive, mechanisms that require further studies for validation. Our methylation assessments are also subject to limitations. It is not clear when psychotherapy-associated DNA methylation changes become stable, and how long such changes may take to produce downstream effects on neurobiology and behaviour. Methylation analysis was necessarily performed using surrogate tissues, which may not reflect epigenetic changes driving neuronal processes such as learning, memory and emotional processing (Kumsta, 2019). Nevertheless, cross-tissue analyses suggest that the methylation profile of saliva correlates more closely with brain tissue than blood does (Braun et al., 2019; Smith et al., 2015). We are also unable to account for the potential effects of certain biological i.e. saliva sample cell type composition, or participant-level factors e.g. diet, smoking and medication, on our methylation values. The relatively long follow-up period in this study, during which participants returned to communities with high violence, increase the likelihood of our results being confounded by environmental influences in the post intervention period. We chose to focus on male participants as males, particularly between the ages of fifteen and 29 years, are most likely to be both victims and perpetrators of violence in South Africa (Seedat et al., 2009). However, as sex can influence the development of both AA and PTSD, as well as epigenetic regulation of behaviour (Augsburger et al., 2017; Baumbach and Zovkic, 2020; Seligowski et al., 2019; Weierstall et al., 2011), our findings may not be applicable to females.

This study has several strengths. As trauma-induced AA is long-lasting and can develop in a self-perpetuating cycle, studies examining therapeutic interventions for AA and PTSD are valuable in mitigating some of the harmful effects of violence and trauma in individuals and communities. This study entailed a carefully considered and rigorously implemented intervention with clinical data collected by research staff with specific training in the field of trauma. Contrary to previous studies conducted in post conflict contexts, the current research assessed interventions in participants who have a history of high trauma exposure and continue to live under a constant sense of threat in communities with ongoing violence and trauma (Hinsberger et al., 2017, 2016; Kaminer et al., 2018), and is thus relevant to other chronic stress scenarios, such as ongoing conflict or mass displacements of people (Kaminer et al., 2018). This is the first study to assess therapy epigenetics in relation to AA and PTSD. The study made use of a considerably longer follow-up timeframe compared to other therapy epigenetic studies, which typically assess methylation immediately after the completion of treatment or after a three-month follow-up period (Schiele et al., 2020). Longer assessment windows are valuable in determining the longevity of the interventions, and the maintenance of benefits. We investigated methylation in a panel of genes that have previously been implicated in psychiatric disorders, a quasi-targeted approach that goes beyond single candidate gene methylation studies. In contract to some of the larger methylation array methods, the EpiTect

methyl array also distinguishes between 5-methylcytosine and 5-hydroxymethylcystosine; two modifications that have opposite effects on expression. This discrimination allows more confidence in inferring reduced expression from increased methylation signal. Finally, this study contributes to the emerging field of therapy epigenetics, an approach that harnesses techniques in psychiatry and molecular biology to identify the biological basis of interindividual differences in psychopathology (Kumsta, 2019). Through such approaches, we can further our understanding of the biological mechanisms underlying aggressive and violent behaviour in order to predict treatment response and monitor therapeutic efficacy. This targeting and tailoring of treatment based on likely effectiveness not only aligns with the drive towards personalised medicine, but could also make an important contribution to reducing the treatment gap in psychiatry and ultimately decreasing the cycle of violence (Schiele et al., 2020).

Our study supports the value of FORNET in reducing symptoms of PTSD and AA. We identified DNA methylation signatures that mapped to the improvement in PSS-I scores in participants receiving FORNET. Though the underlying mechanisms are speculative, previous studies support the involvement of synaptic plasticity and dopaminergic mechanisms (via AUTS2 and NR4A2 respectively) in PTSD. Basic science research also indicates a role for neuroenergetics, which is influenced by TFAM, in aggressive behaviour. Exposure to trauma and violence are common and the consequences far reaching. Our findings, though preliminary, add to the body of therapy epigenetics research, a field which seeks to provide insight into biological mechanisms underlying psychotherapeutic efficacy and can be used to guide future investigations.

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Data statement

Data generated by this study is available upon reasonable request.

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Author statement

Khethelo Xulu: Investigation, Writing – Review & Editing. **Jacqueline Womersley:** Formal analysis, Writing – Original Draft, Writing – Review & Editing, Visualization. **Jessica Sommer:** Investigation, Writing – Review & Editing. **Martina Hinsberger:** Investigation, Writing – Review & Editing. **Thomas Elbert:** Conceptualization, Supervision, Writing – Review & Editing; Funding acquisition. **Roland Weiertsall:** Supervision, Writing – Review & Editing. **Debbie Kaminer:** Supervision, Writing – Review & Editing. **Stefanie Malan-Müller:** Supervision, Writing – Review & Editing. **Soraya Seedat:** Conceptualization, Supervision, Writing – Review & Editing; Funding acquisition. **Sian Hemmings:** Conceptualization, supervision, Writing – Review & Editing.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Figure legends

Figure 1. Longitudinal AAS and PSS-I scores within treatment groups. A) AAS scores declined significantly from baseline to sixteen-month follow-up in participants receiving FORNET ($p = 0.037$). B) PSS-I scores reduced significantly from baseline to eight-month follow-up in participants receiving FORNET ($p = 0.043$). * Significantly different to FORNET at baseline. Data are displayed as mean and standard deviation. AAS = Appetitive Aggression Scale, CBT = Cognitive Behavioural Therapy, FORNET = Narrative Exposure Therapy for Forensic Offender Rehabilitation, PSS-I = PTSD Symptom Scale – Interview, WL = waitlisted.

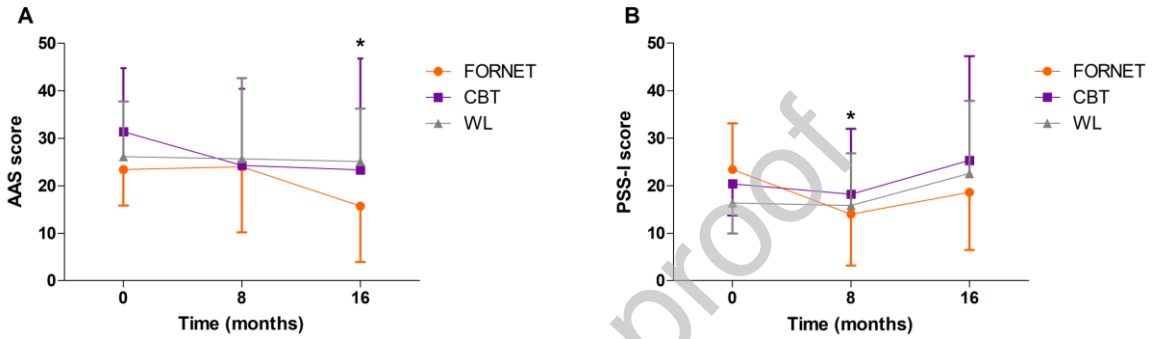

Figure 2. PSS-I scores in participants receiving FORNET are significantly associated with *AUTS2* and *NR4A2* methylation. A) Change in PSS-I score from baseline to both 8- and 16-month follow-up were significantly associated with *AUTS2* methylation over the same period ($p = 0.023$ [8 months] and $p = 0.037$ [16 months]). B) The change in PSS-I score from baseline to 8 months was significantly associated with *NR4A2* methylation ($p = 0.049$). * DNA methylation significantly different to measures at baseline. Data are displayed as mean and standard deviation of z-transformed values. *AUTS2* = autism susceptibility candidate 2; *NR4A2* = nuclear receptor subfamily 4, group A, member 2; PSS-I = PTSD Symptom Scale – Interview

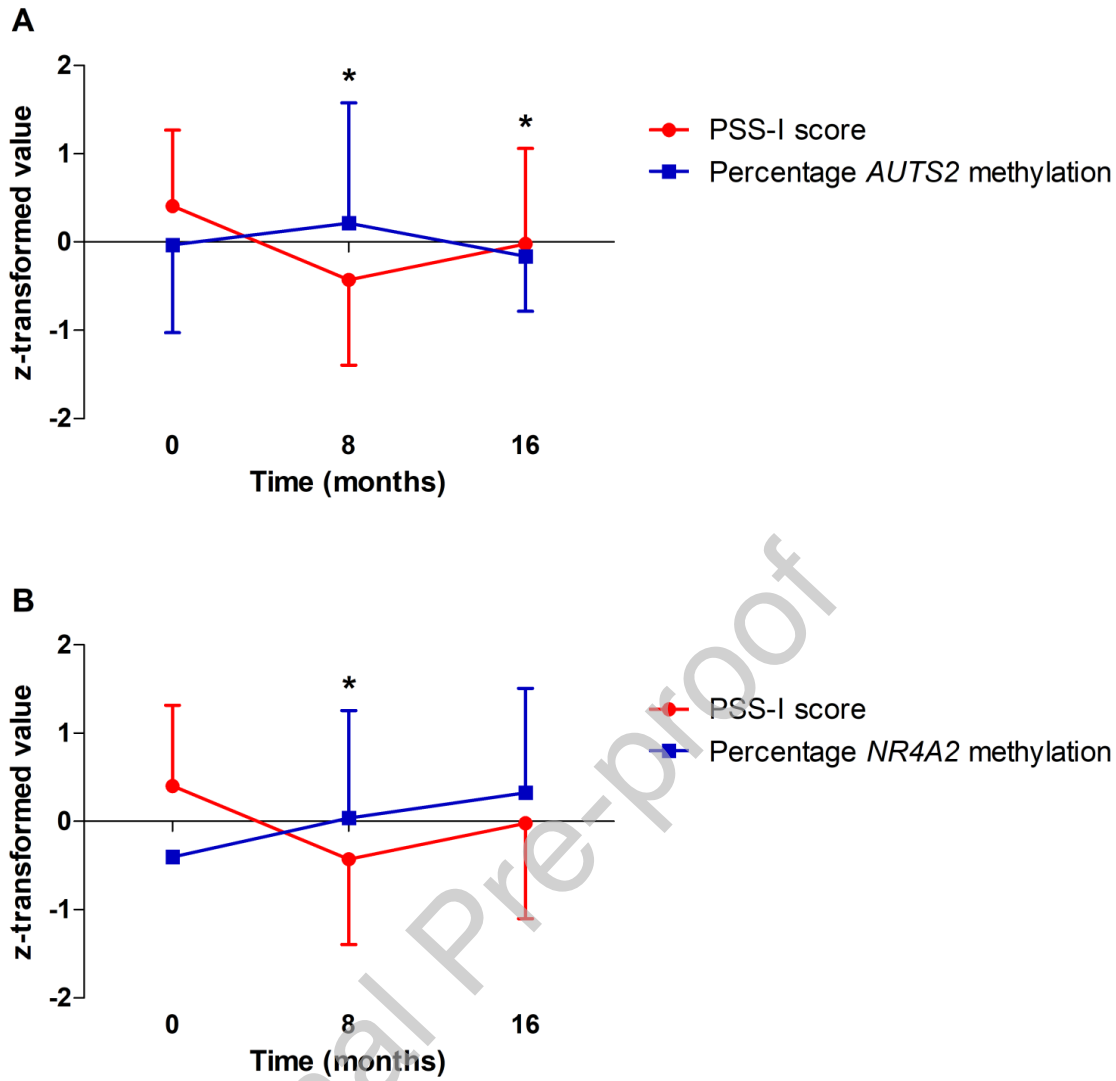

Figure 3. AAS scores and *TFAM* methylation are positively associated. Mixed model analyses using longitudinal data across groups with participant as a random factor indicated that an increase in *TFAM* methylation was associated with an increase in AAS scores ($p = 0.026$).

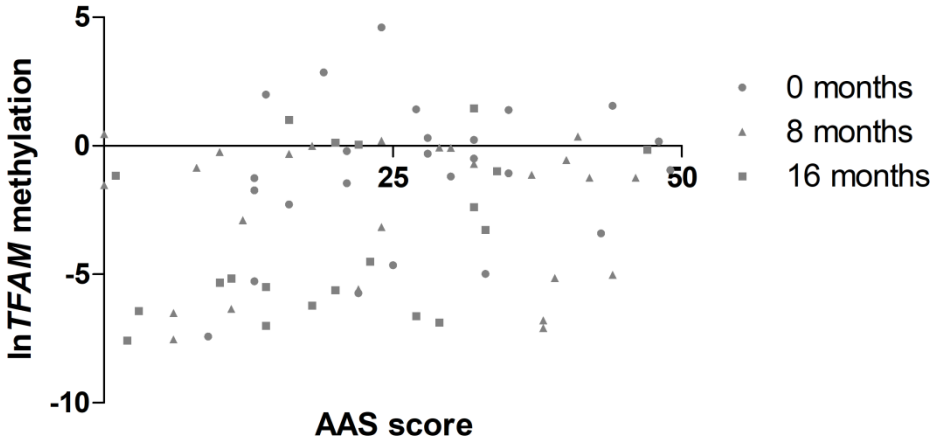

Table 1: List of genes in the EpiTect Methyl II Signature PCR Array (22) for Human Mental Disorders

Gene symbol	Gene name	Reference sequence	Chromosome	Assay chromosome location
APP	amyloid beta (A4) precursor protein	NM_201414	21	27543083
AUTS2	autism susceptibility candidate 2	NM_001127231	7	69062848
AVP	arginine vasopressin	NM_000490	20	3063385
BDNF	brain-derived neurotrophic factor	NM_001709	11	27721819
COMT	catechol-O-methyl transferase	NM_000754	22	19929342
DRD2	dopamine receptor 2	NM_016574	11	113345881
GABRA2	gamma-aminobutyric acid receptor subunit alpha-2	NM_000807	4	46392308
GAD1	glutamate decarboxylase	NM_013445	2	171673862
GLS2	glutaminase 2	NM_013267	12	56882484
HOXA1	homeobox A1	NM_153620	7	27136177
ISL2	islet 2 LIM homeobox	NM_145805	15	76629117
LDLR	low-density lipoprotein receptor	NM_001195803	19	11201186
LHX5	LIM homeobox 5	NM_022363	12	113909800
MECP2	methyl CpG binding protein 2	NM_004992	X	153362836
NR4A2	nuclear receptor subfamily 4, group A, member 2	NM_006186	2	157190532
RELN	Reelin	NM_173054	7	103630017
RPL39	ribosomal protein L39	NM_001000	X	118925617
RPP21	protein subunit of nuclear ribonuclease 2	NM_024839	6	30312999
SMS	spermine synthase	NM_004595	X	21959275
SORBS3	sorbin and sh3 domains-containing protein 3	NM_005775	8	22408886
TERT	telomerase reverse transcriptase	NM_198253	5	1295423
TFAM	transcription factor A, mitochondrial	NM_003201	10	60145034

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Table 2: Descriptive statistics for appetitive aggression and posttraumatic stress disorder symptom severity in the three treatment groups across time

	Baseline			8-month follow-up			16-month follow-up		
	FORNET (*n* = 10)	CBT (*n* = 10)	WL (*n* = 9)	FORNET (*n* = 9)	CBT (*n* = 10)	WL (*n* = 6)	FORNET (*n* = 10)	CBT (*n* = 3)	WL (*n* = 9)
Mean	23.40	31.40	26.11	24.00	24.30	25.66	15.70	23.33	25.11

AAS (SD)	(7.56)	(13.36)	(11.69)	(13.82)	(16.13)	(17.00)	(11.79) *	(23.50)	(11.12)
Mean PSS-I (SD)	23.40 (9.70)	20.40 (6.67)	16.33 (6.40)	14.00 (10.86) *	18.22 (13.72)	15.83 (10.94)	18.60 (12.14)	25.33 (21.96)	22.56 (15.30)

AAS = Appetitive Aggression Scale, CBT = cognitive behavioural therapy, FORNET = narrative exposure therapy for forensic offender rehabilitation, PSS-I = PTSD Symptom Scale - Interview, SD = standard deviation, WL = waitlisted. * significantly different to baseline