Comprehensive Psychiatry 123 (2023) 152381

Contents lists available at ScienceDirect

Comprehensive Psychiatry

journal homepage: www.elsevier.com/locate/comppsych

Acute nicotine exposure blocks aromatase in the limbic brain of healthy women: A [^{11}C]cetrozole PET study

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ARTICLE INFO

Keywords:
Addiction
Aromatase
Brain
Nicotine
PET
Women

ABSTRACT

Background: Of interest to women's mental health, a wealth of studies suggests sex differences in nicotine addiction and treatment response, but their psychoneuroendocrine underpinnings remain largely unknown. A pathway involving sex steroids could indeed be involved in the behavioural effects of nicotine, as it was found to inhibit aromatase *in vitro* and *in vivo* in rodents and non-human primates, respectively. Aromatase regulates the synthesis of oestrogens and, of relevance to addiction, is highly expressed in the limbic brain.
Methods: The present study sought to investigate *in vivo* aromatase availability in relation to exposure to nicotine in healthy women. Structural magnetic resonance imaging and two [^{11}C]cetrozole positron emission tomography (PET) scans were performed to assess the availability of aromatase before and after administration of nicotine. Gonadal hormones and cotinine levels were measured. Given the region-specific expression of aromatase, a ROI-based approach was employed to assess changes in [^{11}C]cetrozole non-displaceable binding potential.
Results: The highest availability of aromatase was found in the right and left thalamus. Upon nicotine exposure, [^{11}C]cetrozole binding in the thalamus was acutely decreased bilaterally (Cohen's d = −0.99). In line, cotinine levels were negatively associated with aromatase availability in the thalamus, although as non-significant trend.
Conclusions: These findings indicate acute blocking of aromatase availability by nicotine in the thalamic area. This suggests a new putative mechanism mediating the effects of nicotine on human behaviour, particularly relevant to sex differences in nicotine addiction.

1. Introduction

Nicotine use disorder, together with the impact of tobacco smoking, brings along a burden of disease constituting one of the leading causes of preventable death [1]. Despite the decrease in tobacco smoking prevalence over the last decades, smoking tobacco still accounted for about 8 million deaths (1.5 millions of women, about 6% of all deaths) and 200 million disability-adjusted life-years in 2019 alone [2]. Additionally, electronic-cigarettes-driven diseases recently emerged from the increasing popularity of electronic nicotine delivery systems as alternatives to tobacco smoking [3]. Both the positive reinforcing effects of nicotine use and the aversive effects of nicotine withdrawal promote continued use and relapse after cessation, making nicotine dependence very difficult to overcome [4].

Of interest to women's mental health are the sex differences associated with nicotine addiction and treatment response [5,6]. Indeed, women seem to be more resistant to nicotine replacement therapy, experience more relapses, show greater vulnerability for heritability of smoking, and are at greater risk to develop primary smoking-related illnesses (lung cancer, myocardial infarction and deterioration in lung

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https://doi.org/10.1016/j.comppsych.2023.152381

Available online 5 March 2023
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function), though the prevalence of nicotine abuse is greater in men than in women [7]. However, the biological basis of these differences is not well understood.

The cognitive and behavioural effects of nicotine are mediated by nicotinic acetylcholine receptors (nAChRs), but additional evidence suggests an alternate pathway involving sex steroids [8,9]. Recently, preclinical studies highlighted a deleterious effect of oestradiol on nicotine dependence-related behaviours such as nicotine seeking and anxiety-like withdrawal symptoms [10,11]. Sex and steroid hormones also exert a modulatory role on the rewarding effect of nicotine [12,13], throughout a somewhat complex interplay with the dopaminergic system [14]. In fact, sex differences have been observed in the dopamine release induced by cigarette smoking using positron emission tomography (PET), illustrating a faster dopaminergic response in the dorsal striatum of female smokers, while a consistent and rapid dopamine release in the ventral striatum was only observed in men [15]. Furthermore, likely due to direct effects of nicotine on the ovary, lower levels of oestrogens [16,17] and twice the risk of experiencing premature and early menopause [18] were found in female smokers compared to their non-smoking counterparts, further highlighting the link between nicotine and sex steroids in women, though at a more peripheral level.

Androgens and oestrogens are commonly viewed as the "male" and "female" hormones, respectively, despite the presence of both in human bodies of both sexes. Among the mechanisms able to control the androgen-oestrogen ratio and the oeastradiol synthesis both acutely and chronically is the enzyme aromatase (also named oestrogen synthase) [19]. Aromatase catalyses the conversion of androgens (C_{19} steroids) to oestrogens (C_{18}), *i.e.*, androstenedione and testosterone to oestrone and 17β-oestradiol, respectively, by aromatisation of the A-ring [19]. In humans, aromatase is present in the limbic brain where testosterone and oeastradiol exert their most profound effects, namely the thalamus and the preoptic area, the amygdala, and the medulla, whereas lower levels are found in occipital and temporal cortices, putamen, nucleus accumbens, cerebellum, and white matter [20,21]. Evidence from human post-mortem studies on aromatase mRNA expression corroborates these expression patterns [20,22–25].

Nicotine was found to reduce human aromatase activity and expression *in vitro* [26–28]. Prenatal exposure to nicotine is associated with a reduced aromatase activity as well as mRNA and protein expression in foetal, neonatal and adult rodents [28–30]. Similar observations were made for the nicotine metabolite cotinine [26,29]. Interestingly, in non-human primates, a [^{11}C]vorozole PET study showed that nicotine, in serum concentrations corresponding to those of cigarette smokers, reduces aromatase availability in the amygdala and striatum of female baboons [31]. Taken together, this evidence points to the inhibition of aromatase as an additional pathway by which nicotine can affect cognition and behaviour, particularly where such effects are sexually dimorphic [8], and calls for translational evidence in humans. The depiction of nicotine-aromatase interactions in humans may unveil novel mechanisms mediating the effects of nicotine and open up therapeutic strategies for cigarette smoking/nicotine addiction in women.

The aromatase inhibitor, cetrozole, labelled with carbon-11, allows the investigation of brain aromatase availability *in vivo*. This radiotracer has higher signal-to-noise ratio, selectivity and specificity, as well as improved metabolic stability compared to its predecessor [^{11}C]vorozole [32]. Similar to [^{11}C]vorozole, [^{11}C]cetrozole binds to a single binding site of aromatase, although with a 5.5 times higher affinity (K_d = 0.11 nM) [32].

The aim of this study was to investigate the effect of nicotine on *in vivo* aromatase availability assessed with [^{11}C]cetrozole PET in the brain of healthy women. Nicotine-induced blocking of aromatase was expected in the limbic regions with the highest aromatase levels. Moreover, levels of cotinine, the main nicotine metabolite, were assessed in relation to [^{11}C]cetrozole binding and hypothesized to negatively correlate with aromatase availability.

2. Materials and methods

The present study is part of the on-going Brain Sex Hormones (BSH) project that aims to investigate the effect of endogenous and exogenous sex hormones on brain and behaviour in healthy women. It was carried out at the Department of Women's and Children's Health, Uppsala University Hospital, between 2016 and 2018. Approval has been received from the Regional Ethical Review Board in Uppsala (2014/393). The PET data on which this study is based had solely been used to compare the methodology related to the quantification of [^{11}C]cetrozole binding [33].

2.1. Participants

The participants were recruited by advertisement and assessed at the Department of Women's and Children's Health, Uppsala University Hospital. Thirteen Swedish speaking, heterosexual women (age 22–33) with regular menstrual cycles (25–34 days) were recruited. Participants were excluded if they met the following criteria: current or past (< 6 months) pregnancy, breastfeeding, use of hormonal contraceptive, hormonal or psychoactive drug treatment, nicotine use, neurological disease or psychiatric disorder, high blood pressure (≥ 140/90 mmHg), obesity (BMI ≥ 30 kg/m^2), left-handedness, and contraindications to MRI and PET. Psychiatric disorders were ruled out by the MINI Neuropsychiatric Interview. Prior to scanning, all women underwent a urine pregnancy test. In addition, participants were required to not have eaten for two hours, not have taken medications, consumed caffeine or alcohol for twelve hours before the scanning session, and to have had a good night of sleep. Three women were excluded from the study due to incomplete PET data and dropout, resulting in ten participants.

2.2. Procedure

Two neuroimaging scans were performed (*i.e.*, baseline and nicotine challenge), as illustrated in Fig. 2a. The sessions were scheduled one menstrual cycle apart (maximum ±3 days), to ensure a similar hormonal milieu. Due to some technical issues, one participant could not be scanned twice in the same menstrual cycle phase (day 6 at baseline, day 27 for the nicotine challenge). Venous blood samples were collected at the beginning of the two sessions to measure the levels of sex steroids. Monitoring of the menstrual cycle phase was based on self-reports on menstrual cycle length, previous menstrual bleeding onset, and confirmed by the onset of the next menses, as well as serum progesterone and oeastradiol levels. A description of the menstrual cycle timing and gonadal hormones levels for each session is provided in supplementary Table 1.

During the second session, within five minutes before the scan, 0.5 mg of Nicorette® nasal spray were administered in each nostril, to mimic the dose of nicotine contained in one cigarette. For ethical reasons, higher doses could not be administered to healthy women. Previous studies have shown that the highest absorption of nicotine that is delivered *via* 2 mg nasal spray occurs during the first 5 min after exposure, comparable to smoking a cigarette [34]. The estimated mean dose of nicotine reaching systemic circulation in our study was 0.007 mg/kg. Systemic nicotine levels were expected to peak 5 to 10 min after administration of the 1 mg nasal spray, and slowly decrease to reach a relatively stable concentration of 2 ng/ml at the end of the PET scan [35].

Saliva samples were collected right before nicotine administration and after the scan (95 min after exposure). Due to the duration of the PET-scan and the short half-life of nicotine (about two hours) compared to its metabolite cotinine (about 16 h) [36], salivary cotinine levels were measured as a proxy of nicotine use status and nicotine absorption following exposure.

2.3. Hormone and cotinine analyses

The concentrations of oestradiol, progesterone, and testosterone were measured at the Core Facility of Metabolomics, University of Bergen, by liquid chromatography – tandem mass spectrometry. Cotinine concentrations were measured by Truly Labs, Lund, Sweden using the Enzyme-Linked Immuno Sorbent Assay (ELISA) kit from Salimetrics, USA [37].

2.4. Neuroimaging data acquisition

Brain aromatase availability was assessed during a dynamic 90-min PET scan of 26 frames of increasing length (6 × 10, 3 × 20, 2 × 30, 3 × 60, 2 × 120, 4 × 300, 6 × 600 s) starting with the bolus injection of the [^{11}C]cetrozole, a non-steroidal ^{11}C-radiolabelled reversible aromatase inhibitor with higher signal-to-noise ratio, specificity and affinity than [^{11}C]vorozole [32]. Synthesis of [^{11}C]cetrozole and PET scanning was performed as described by Jonasson, Nordeman [33]. Briefly, The PET scans were acquired using either a Discovery ST, Discovery IQ, or Discovery MI PET/CT scanner (GE Healthcare, Milwaukee, WI, USA). [^{11}C]cetrozole PET image reconstruction settings were set to yield similar spatial resolution (about 6 mm) across the three scanners used: Ordered Subsets Expectation Maximization with two iterations and 21 subsets and a 4-mm postprocessing filter for the Discovery ST, four iterations and 12 subsets and a 4-mm postprocessing filter for the Discovery IQ, and three iterations and 34 subsets and a 5-mm postprocessing filter for the Discovery MI. At each PET scanning session, participants received 4 MBq kg^{-1} of [^{11}C]cetrozole, to a maximum of 400 MBq. The sessions included a structural magnetic resonance imaging (sMRI) scan for anatomical information, acquired on a 3 T Achieva dStream scanner (Philips Healthcare, Best, The Netherlands) at the Department of Radiology, Uppsala University Hospital.

2.5. PET data analysis and preprocessing

The dynamic [^{11}C]cetrozole PET images were corrected for subject movements using the VOIager software (GE Healthcare, Uppsala, Sweden). Co-registration of the MRI scans to the PET images was carried out based on a 6-parameter rigid transformation using SPM8 (Wellcome Trust Center for Neuroimaging, University College London, UK). Regions of interest (ROIs) were defined using a probabilistic VOI template implemented in PVElab [38], and projected over all frames of the dynamic PET data to obtain time–activity curves (TACs). For the amygdala, left and right ROIs were defined using a 70% isocontour mask drawn on the [^{11}C]cetrozole parametric maps.

Non-displaceable binding potential (BP_{ND}) was calculated as the distribution volume (V_T) ratio between the target and reference regions minus one (DVR-1), using the Logan reference tissue model (LRTM) with the cerebellum grey matter as reference region [39]. LRTM DVR-1 was estimated at each voxel to generate parametric images of the [^{11}C]cetrozole binding, using an in-house developed software in Matlab (The Mathworks, Natick, USA).

Preprocessing of [^{11}C]cetrozole scans was carried out in SPM12. DVR-1 BP_{ND} maps were normalized to standard MNI space using the deformation fields resulting from the normalization of the co-registered MRI scans to the MNI template. The warped BP_{ND} maps were smoothed using a 10 mm full width-half maximum (FWHM) filter.

2.6. Statistical analyses

For the statistical analyses, brain regions with BP_{ND} values $\geq$0.3 were identified as areas with reliable binding of the tracer, based on previous reports on [^{11}C]cetrozole PET imaging in humans and non-human primates [32]. ROI analyses included the thalamus, amygdala, and hypothalamus. A 2-way repeated-measures ANOVA was performed to assess ROI-by-treatment interaction effects on [^{11}C]cetrozole BP_{ND}. Wilcoxon signed-rank tests comparing the mean BP_{ND} inside ROI between the baseline and nicotine sessions were run in Statistical Package for the Social Sciences (SPSS for Windows, version 26). Significance was set at $q < 0.1$, False Discovery Rate (FDR)-corrected for multiple testing across ROIs. Between-sessions differences in relative menstrual cycle day at scanning, and serum gonadal hormones were assessed using paired-samples Wilcoxon rank tests in SPSS, version 26.

One subject's BP_{ND} values in the thalamus showed significant deviation from the others' (> 2 SD), thus she was excluded from the analyses involving the thalamus ($n = 9$). To assess the influence of menstrual cycle timing on the findings, the PET analyses were repeated while excluding the participant scanned in two different menstrual cycle phases ($n = 8$ for the analyses involving the thalamus, n = 9 for the amygdala and hypothalamus analyses). Likewise, in order to assess the influence of recent nicotine exposure on the findings, the analyses were repeated while excluding the two participants for which cotinine levels before exposure to nicotine spray were above 60 ng/ml. As one of these two participants was already excluded from the main analysis as an outlier in thalamic BP_{ND} values, all these tests included eight women.

Associations between [^{11}C]cetrozole binding and nicotine absorption, as assessed by cotinine concentration, following exposure to nicotine nasal spray were investigated through correlation analyses. These analyses included seven women (excluding one participant who did not provide a saliva sample after the scan, and two participants who recently used nicotine prior to the study). Spearman correlations between the salivary concentration of cotinine measured after the scan and the mean BP values of the thalamus ROI in the nicotine challenge session, were carried out in SPSS, version 26.

3. Results

3.1. Participants

In total, ten healthy women were scanned at baseline and after nicotine exposure. These ten participants (22–33 year-old) were heterosexual, with an education level equal to or higher than high school (14.9 years of education on average). The majority (70%) was studying, while 30% worked full time. Two participants self-reported to have suffered from depression in the past and recovered at least 6 months before the study. Only one participant had given birth, the remaining women were nulliparous. Across all participants, no significant difference in the relative menstrual cycle day, or oestradiol and progesterone levels between sessions was found (Table S1). All participants reported to be non-smokers. However, salivary cotinine levels indicated that two participants had used nicotine products prior to the study (concentration > 60 ng/ml). Except for these two participants, salivary cotinine levels at baseline were all below the detection threshold of 0.8 ng/ml. Administration of nicotine was associated with salivary cotinine concentrations ranging from about 8 to 18 ng/ml (mean ± SD: 13 ± 4 ng/ml) after the PET scan.

3.2. Effect of nicotine on aromatase

At baseline, specific binding of [^{11}C]cetrozole was found in the thalamic area, hypothalamus and amygdala (Fig. 1), with the highest levels in the thalamus. We found a significant ROI-by-session interaction effect on [^{11}C]cetrozole BP_{ND} ($F = 6.40$, $p = 0.026$, $\eta_p^2 = 0.65$), as highlighted by a 2-way repeated measure ANOVA. This finding was driven by reduced BP_{ND} values in the thalamus following acute nicotine administration ($p = 0.021$, $q = 0.063$, Cohen's $d = -0.99$, Fig. 2b). Overall, eight out of the nine participants included in the analysis displayed a decrease in [^{11}C]cetrozole binding in the thalamus (Fig. 2b, ranging from 2 to 36% decrease). No nicotine-induced effects were noted in the other two ROIs, the amygdala ($p = 0.575$, $q = 0.862$, Fig. 2b) and the hypothalamus ($p = 0.721$, $q = 0.721$, Fig. 2b).

Upon arterial blood sampling availability on both PET scans,

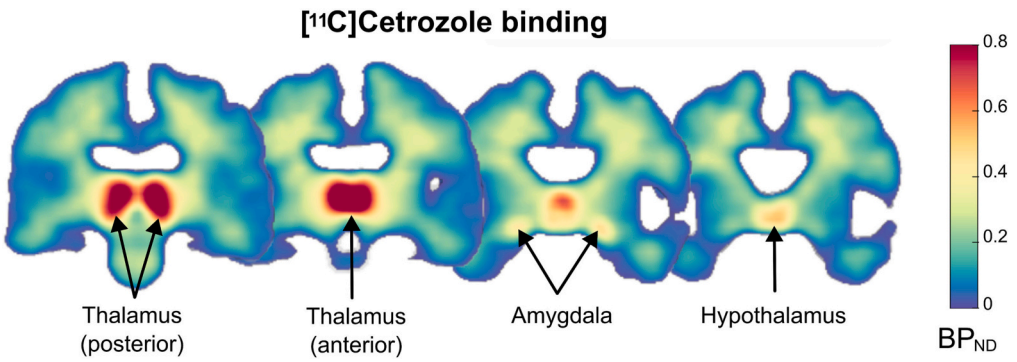

Fig. 1. Distribution of [^{11}C] cetrozole binding in the brain of healthy women. Parametric map of [^{11}C]cetrozole non-displaceable binding potential (BP_{ND}), averaged across all participants ($N = 10$). Coronal sections of the brain (y = −24, −18, −6, −2) show the highest aromatase availability in the thalamus, followed by the amygdala and hypothalamus.

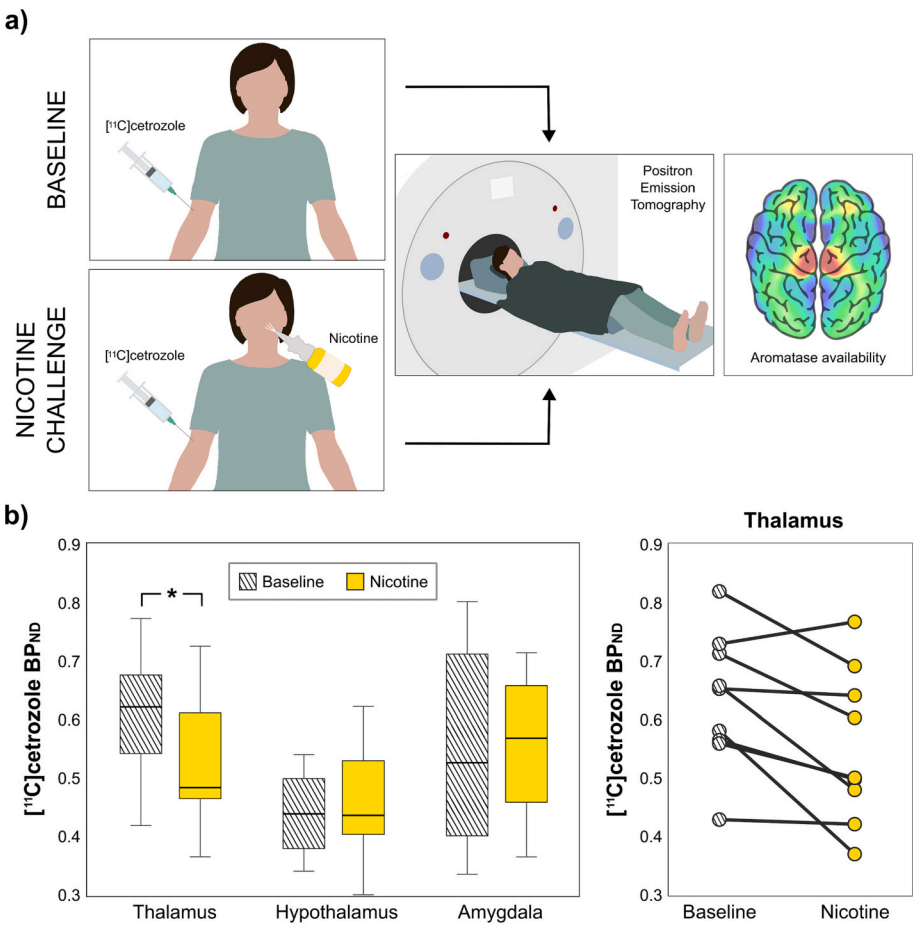

Fig. 2. Effect of acute nicotine exposure on aromatase availability across ROIs. a) Study design. Each participant underwent 90-min PET imaging twice with the [^{11}C]cetrozole radiotracer targeting aromatase, once at baseline and once following an acute nasal administration of nicotine. The two sessions were scheduled to fall in the same menstrual cycle phase, to avoid any bias due to differential hormonal milieu. **b)** Left panel: Box plot of [^{11}C] cetrozole non-displaceable binding potential (BP_{ND}) estimates at the baseline and nicotine sessions inside regions-of-interest (ROIs). Right panel: Individual pattern of BP_{ND} change between the baseline and nicotine PET sessions, in the Thalamus ROI. Thalamus BP_{ND} plots exclude one outlier ($N = 9$).

graphical inspection of [^{11}C]cetrozole volume of distribution (V_T) within ROIs and the reference region (cerebellum) highlighted a similar pattern of aromatase availability reduction after the nicotine challenge. The effect seen in the thalamus could also be seen for the hypothalamus, the amygdala, and the cerebellum, as illustrated in supplementary Fig. 1, representing the V_T values from one participant for which arterial sampling data was available.

The findings remained unchanged when we excluded the participant that was scanned in two different menstrual cycle phases (Baseline > Nicotine in the thalamus, $p = 0.036$). Similarly, excluding the two participants with high cotinine levels from the analysis did not affect the results (Baseline > Nicotine, in the thalamus, $p = 0.036$).

3.3. Relationship between nicotine absorption and aromatase availability

In the nicotine challenge session, we observed a pattern illustrating a negative correlation between salivary cotinine concentrations and [^{11}C] cetrozole BP_{ND} values within the thalamus, although without reaching statistical significance ($p > 0.1$) (Fig. 3).

4. Discussion

For the first time, we provide indication of an acute blocking effect of nicotine on aromatase availability in the human brain, especially in the area where the highest level of aromatase binding is found, *i.e.*, the

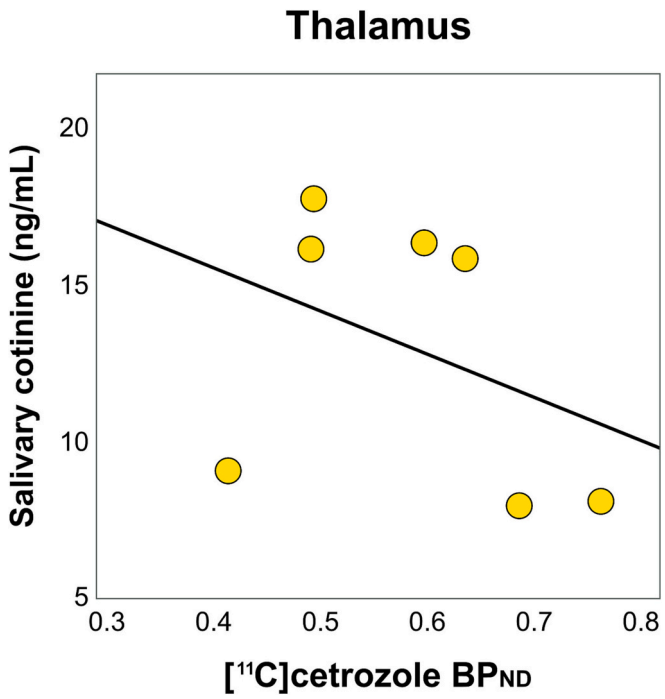

Fig. 3. Association between aromatase availability in the thalamus and salivary cotinine levels. Scatter plot representing the relationship between aromatase availability in the Thalamus and salivary cotinine levels following the nicotine challenge (second PET session). After exclusion of the two participants displaying high cotinine levels at baseline and the one outlier presenting aberrant BP_{ND} values in the Thalamus, a pattern depicting a negative correlation between the two measures emerged ($N = 7$). The pattern suggests that higher levels of cotinine relate to lower aromatase availability in the Thalamus. This relationship however did not reach statistical significance.

thalamus. More specifically, the study shows a decrease in aromatase binding in the thalamus of healthy women following acute nicotine administration, indicating a large effect (d = −0.99). This result is discussed in relation to the sex-related differences in smoking and nicotine dependence [5,6].

4.1. Translational evidence of aromatase blockade by nicotine

The present findings translate evidence of aromatase blocking by nicotine, as observed *in vivo* in non-human primates [31]. In baboons, the highest signal from the radiotracer was found in the amygdala and was reduced after injection of nicotine (0.015 mg/kg) [31]. In regions such as the preoptic area and the striatum, where the radiotracer signal was lower than in the amygdala, only a larger dose of nicotine (0.03 mg/kg) led to a significantly reduced uptake of the radiotracer [31]. As the present study confirms, the thalamus is the brain region with the highest aromatase availability in women [21,33,40]. Therefore, with an estimated mean dose of nicotine reaching systemic circulation of 0.007 mg/kg in this study, it was expected that small though significant effects would be found in this region, as confirmed by our findings. For ethical reasons, higher doses could not be administered to healthy women. It is indeed likely that, in the presence of a higher dose of nicotine, a reduction of [^{11}C]cetrozole binding could also be observed in regions with lower aromatase availability, such as the amygdala and hypothalamus, in line with the study conducted in non-human primates [31]. This is further supported by the observed reduction in V_T values across the ROIs and the reference region (cerebellum) following the nicotine challenge in the subject for which arterial blood sampling was available at both time points (Fig. S1). The presence of low amounts of aromatase in the cerebellum, together with the reduced radioactivity elicited by nicotine exposure within this region, suggest that the effect of nicotine on aromatase as assessed by [^{11}C]cetrozole BP_{ND} was here underestimated. In line, Biegon and colleagues also graphically described lower levels of aromatase availability in the human brain of few heavy smokers ($n = 4$) in comparison with non-smokers [21]. Also, important to consider, tobacco smokers are chronically exposed to a much higher dose of nicotine, which might explain the difference between our findings and those obtained in a small group of heavy smokers [21].

4.2. Nicotine metabolism and aromatase availability

To assess the effect of acute nicotine exposure in healthy women, nasal administration of nicotine was here employed, as it is the fastest and most direct way for nicotine to access the brain, through the olfactory bulb [41]. Among nicotine replacement products, nicotine nasal spray is the only one to provide a rapid delivery of nicotine to the brain, that is closer to the rate of nicotine delivery achieved with smoking [41]. In fact, the pattern of plasma nicotine absorption from nasal sprays is highly similar to that of cigarette smoking, although the magnitude of the resulting increase in nicotine concentrations is much lower [35]. Following administration of Nicorette® nasal spray, approximately half of the nicotine enters the systemic circulation [42], meaning that each participant is expected to have absorbed a dose of about 0.5 mg.

Nicotine has a rather short half-life of about 120–150 min, while its metabolite cotinine is eliminated much slower, with a half-life of 720–1200 min [36]. Cotinine concentration progressively increases to reach a peak concentration of about 24 ng/ml around 180 min post-smoking, with very similar plasma and saliva measurements [43]. Thus, we expected relatively high levels of cotinine in the saliva samples collected 95 min after exposure to the nicotine spray. Accordingly, salivary cotinine concentrations measured after the nicotine challenge scan reached 13 ng/ml on average, in the non-tobacco users. Interestingly, the variation in cotinine levels negatively correlated with aromatase blockade in the thalamus as a non-significant trend – in line with previous evidence pointing to a dose-dependent effect of nicotine on aromatase availability in female baboons [31].

4.3. Region-specific expression of aromatase and effect of nicotine

Aromatase expression is species-, and region-specific. In rodents, the highest expression of aromatase is found in the amygdala, followed by the bed nucleus of the stria terminalis and the preoptic area [25,32,44]. In non-human primates, *in vivo* PET-studies revealed the highest levels of aromatase in the amygdala, slightly lower levels in the hypothalamus/preoptic area, with low amounts in the basal ganglia, cortex, white matter and cerebellum [31,32,45–48]. Previous studies conducted in humans, on the other hand, repeatedly found the highest expression of aromatase in the thalamus, lower but clear expression in the amygdala, hypothalamus/preoptic area and medulla, and low but detectable amounts in white matter, putamen nuclei and cerebellum [20,33,40]. Thus, based on the observation that higher doses of nicotine are necessary to inhibit aromatase in the regions where it is moderately or sparsely expressed in non-human primates [31], a reduction of [^{11}C]cetrozole binding was here expected in the thalamus of healthy women exposed to nicotine nasal spray.

The thalamus has long been solely considered as a sensory relay centre, although only a few of the thalamic nuclei receive inputs from sensory receptors and send information to primary sensory cortices [49]. In fact, considering that each thalamic nucleus displays a unique pattern of connections with cortical and subcortical brain areas, it is likely that the different thalamic nuclei and sub-nuclei play a specific role in sensory, motor, cognitive, and limbic functions [49]. Interestingly, the thalamus has been suggested to be involved in nicotine dependence, as illustrated by functional MRI studies showing an increased reactivity of this region in relation to acute nicotine exposure, smoking cues, and craving in smokers [50,51]. In fact, the highest expression of nAChRs in the human brain is found in the thalamus [52], and a widespread

upregulation of nAChRs has been demonstrated in smokers compared to controls [53]. Moreover, smaller grey matter volume in the thalamus has been reported in smokers compared to their non-smoking counterparts [54–56], further highlighting the involvement of this region in nicotine addiction, and the relevance of the present findings to the sex differences reported on this disorder [5,6].

Considering the acute exposure to nicotine in the present study, it is likely that our findings reflect a direct blockade of its enzymatic activity. This is in line with early *in vitro* experiments showing that aromatase activity inhibition by nicotine is blocked by supraphysiological amounts of androstenedione, indicating a competition between aromatase substrate and nicotine [26]. Of relevance to nicotine dependence and long-term effects of nicotine, recent translational evidence obtained from *in vitro* and *in vivo* experiments conducted on human and rodent ovarian tissues indicates that the effects of nicotine on aromatase expression may additionally be mediated by an increased expression of nAchRs and concomitant epigenetic changes in the aromatase promotor region [28]. Thus, we hypothesize that both acute and secondary regulatory mechanisms involving nicotine and aromatase might participate in the sex differences observed in nicotine dependence phenotypes [5,6].

As many aromatase effects are mediated by its product oestrogen, it is noteworthy that the presence of oestrogen receptors, especially the ERβ subtype, has been noted in the human thalamus post-mortem [57]). Cognitive or behavioural effects of varying oestrogen levels in this area have not been studied but are expected, considering structural and functional connections of the hypothalamic-pituitary system with the thalamus.

4.4. Inter- and intrapersonal variations in aromatase expression

Sex differences in aromatase availability have been previously reported, suggesting higher levels of expression in males compared to females, both in rodents [25,32,58,59] and humans [21,40], though other studies did not find significant differences, both in rodents [60] and humans [22,61,62]. In line with this, it would be relevant to further investigate whether the observed inhibitory role of nicotine on aromatase is sex-specific or not, as the present study did not include male participants. As regards variations in aromatase availability in the human brain throughout the menstrual cycle, present knowledge is insufficient to draw any conclusion. To date, only one study including three women reported on the matter, indicating no significant variations in aromatase availability between high-oestrogen and low-oestrogen phases [20]. Evidence from animal studies is also limited and inconclusive. For instance, one *in-vivo* PET study based on three female baboons suggests that aromatase availability may vary according to their menstrual cycle, as illustrated by a lower availability in the high-oestrogen phase compared to low-oestrogen phases [47]. In rodents, early *in vitro* evidence indicates no variations in aromatase activity within the hypothalamus/preoptic area throughout the oestrous cycle [63]. In the present study, excluding the one participant who was not scanned in the same menstrual cycle phase for both sessions did not impact the findings.

4.5. Strengths and limitations

While it is technically challenging to measure local concentration of androgens and oestrogens in the brain, and even more to assess the turnover of these hormones, the enzyme aromatase can be seen as a lens on these dynamics. Furthermore, *in vivo* assessments of aromatase enabled by PET imaging in healthy individuals go beyond pre-clinical and post-mortem studies, providing a potential proxy of the androgen-oestrogen dynamics related to sex differences in brain structure and function, behaviour, as well as nicotine addiction. Among the strengths of the study is the use of [^{11}C]cetrozole, which provides high signal-to-noise ratio, selectivity and specificity, as well as metabolic stability compared to its predecessor [^{11}C]vorozole [32].

Nevertheless, this study included a small group of participants, resulting in a moderate statistical power ($1 - \beta$ error probability = 0.71), which entails only large effects being able to be detected. The present findings therefore call for replication in a larger population to confirm the region-specific effect of nicotine on aromatase in the human brain, and to explore potential sex differences. Likewise, future studies following up healthy controls in comparison with nicotine users and nicotine-dependent individuals could shed light on the long-term effect of nicotine exposure and cessation on central aromatase levels.

5. Conclusions

The present study shows the highest [^{11}C]cetrozole binding in the thalamus, thus confirming the pattern of distribution of aromatase in the human brain. Furthermore, this work highlights the inhibitory effect of nicotine on aromatase availability *in vivo* in the thalamus of the healthy female brain, following an acute exposure to nicotine nasal spray. These findings suggest a new putative mechanism mediating the effects of nicotine on human behaviour, particularly where such effects are sexually dimorphic. While a wealth of studies points to sex- and hormone-related differences in behaviour and psychiatry, their psychoneuroendocrine underpinnings remain largely unknown [64–66]. Thus, the role of aromatase in sexually dimorphic behaviours and related impairments in humans has not been established, although it is suspected to be involved in cognition, aggression, personality, mood, and mental illness [67]. Future work following up on these aspects would likely improve our understanding of the sex-specific clinical profile of nicotine dependence and help move toward more personalized and efficient treatment strategies for women who struggle with smoking cessation.

Declaration of Competing Interest

None.

Acknowledgements

The author would like to thank the subjects who participated in this study, the research nurses Kaisa Lyon and Mimmi Lidholm, Lena Moby at the KBH unit, Britt-Marie Bolinder at the MR center, Patrik Nordeman, Jonas Eriksson and Helena Wilking at the PET center at Uppsala University Hospital, Hanna Lif, Julia Breedh, and the students of the Neuropsychopharmacology group, Dr. Jean Pettersson at Uppsala University for quantification of palladium by ICP-AES, as well as Prof. Simon S. Hustad, for the quantification of hormones concentrations. The study was supported by the ALF and SciLifeLab. The funding sources were not involved in designing the study, the collection, analysis and interpretation of data, the writing of the report, and in the decision to submit the manuscript for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.comppsych.2023.152381.

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