Region-specific up-regulation of oxytocin receptor binding in the brain of mice following chronic nicotine administration

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Abstract

Nicotine addiction is considered to be the main preventable cause of death worldwide. While growing evidence indicates that the neurohypophysial peptide oxytocin can modulate the addictive properties of several abused drugs, the regulation of the oxytocinergic system following nicotine administration has so far received little attention. Here, we examined the effects of long-term nicotine or saline administration on the central oxytocinergic system using [^{125}I]OVTA autoradiographic binding in mouse brain. Male, 7-week old C57BL6J mice were treated with either nicotine (7.8 mg/kg daily; rate of 0.5 μl per hour) or saline for a period of 14-days via osmotic minipumps. Chronic nicotine administration induced a marked region-specific upregulation of the oxytocin receptor binding in the amygdala, a brain region involved in stress and emotional regulation. These results provide direct evidence for nicotine-induced neuroadaptations in the oxytocinergic system, which may be involved in the modulation of nicotine-seeking as well as emotional consequence of chronic drug use.

Keywords: nicotine, oxytocin receptor, amygdala, mice

Highlights

- Nicotine administration increases amygdalar oxytocin receptor binding
- Chronic nicotine does not alter oxytocin receptor binding in the striatum
- Oxytocin may be a potential target for the treatment of nicotine addiction

Introduction

Cigarette smoking is considered the main preventable cause of death worldwide [1]. While there is evidence suggesting that nicotine may exert antidepressant [2-5] and anxiolytic [6] effects, chronic nicotine use has been also associated with severe depression symptoms [7-9] and anxiety, which persist following abstinence [10,11]. This negative affective state following nicotine cessation might contribute to relapse [12]. Although there are currently numerous therapeutic agents and cognitive behavioural interventions **for smoking cessation** that are considered beneficial for the treatment of nicotine addiction, none of these therapeutic strategies has been shown to effectively prevent relapse to nicotine-seeking following abstinence [13]. **In fact, among the 40% of smokers undergoing smoking-cessation interventions, only a small percentage of 4% achieve a long-term abstinence for 6 to 12 months [14]. Therefore, development of an optimal treatment for the effective treatment and prevention of nicotine use and relapse following abstinence requires further understanding of the mechanisms contributing to nicotine long-term abuse, which might be associated with the emergence of emotional impairment during withdrawal.**

Emerging evidence indicates the involvement of the oxytocinergic system in drug addiction processes [15-17]. In particular, chronic administration of addictive substances including cocaine [18,19], methamphetamine [20], opioids [21] and alcohol [22] have been shown to induce marked alterations in the oxytocin (OT) system in the brain, which might be involved in the modulation of the emotional consequences of chronic drug use. Indeed, recent evidence supports an association between oxytocinergic deficiency and the negative emotional consequences of drug addiction, including depression, anxiety and social deficits [21]. OT-producing neurons located in the hypothalamus also project to several brain regions involved in drug-seeking

behaviour as well as emotional regulation, including the septum and amygdala, where oxytocin receptors (OTR) are expressed [23].Few previous studies also demonstrated a role for OT in the modulation of nicotine addiction processes. In particular, acute intravenous administration of nicotine has been shown to decrease OT content in the pituitary of rats [24], and systemic administration of OT abolished physical somatic symptoms of nicotine withdrawal in rats [25]. Overall, although these studies clearly support the involvement of OT in nicotine addiction, the effects of chronic nicotine administration on the central oxytocinergic system remains largely unknown.

Based on the evidence implicating the OT neuropeptidergic system in addictive-related behaviours, we hypothesized that chronic nicotine administration might also induce alterations in the central oxytocinergic system. This is the first study to investigate the effects of chronic nicotine treatment on oxytocin receptor binding with the use of autoradiographic binding.

Materials and Methods

Animals and chronic nicotine administration paradigm

Male C57BL/6J mice (seven-week old, Charles River Laboratories, Kingston, UK), were individually housed in a temperature-controlled environment with a 12:12-hour light/dark cycle (lights on at 06:00). Food and water were available *ad libitum*. Mice were given seven days to acclimatize to their new environment and were handled daily by the experimenter. Mice were treated with a nicotine administration paradigm as described previously [26]. Briefly, saline or nicotine hydrogen salt (7.8 mg/kg/day; Sigma-Aldrich, UK) were administered via osmotic mini-pumps (ALZET®2002 model, Charles River, UK). For minipump implantation, mice were anaesthetised using an isoflurane/oxygen vapour mixture (3.5%–4.5%; Isoflo, Abbott Laboratories Ltd, UK). A single incision along the midline of the back of each animal was made and osmotic mini-pumps were placed in parallel position to the spine. The flow operator was pointing away from the incision site. Nicotine was delivered for a period of 14 days at the daily dose of 7.8 mg/kg (free-base weight), at a rate of 0.5 μl per hour. This dose has been shown to induce blood nicotine levels comparable to the values measured in human smokers [26].

All animal care and experimental procedures complied with protocols approved by the University of Surrey Animal Welfare and Ethical Review Body and by the UK Home Office under Animals (Scientific Procedures) Act 1986. Mice were randomly assigned to two different drug-administration groups; control saline-treated group and chronic nicotine-treated group.

OTR autoradiography

OTR binding was carried out on sections from 14-day saline- and nicotine-treated mice as previously described [20]. Total binding was determined by incubating sections with 50 pM [^{125}I]-ornithine vasotocin (OVTA) for 1 hour in an incubation buffer medium containing 50mM Tris-HCl, 10mM $MgCl_2$, 1mM ethylenediaminetetraacetic acid (EDTA), 0.1 % w/v bovine serum albumin, and 0.05 % w/v bacitracin (Sigma-Aldrich, Poole, UK, pH 7.4 at room temperature). Adjacent sections were incubated with [^{125}I]-OVTA (50 pM) in the presence of 50μM unlabelled (Thr^4,Gly^7)-oxytocin (Bachem, Germany), to determine non-specific binding (NSB). Slides were apposed to Kodak MR-1 films (Sigma-Aldrich, UK) in Hypercassettes with **autoradiographic [^{14}C] microscales of known radioactive concentration (GE Healthcare Life Sciences, Amersham, U.K.)** for 3 days. Films were developed in a 50% Kodak D19 developer solution (Sigma-Aldrich, Poole, UK) and analyzed using MCID image analyzer (Image Research, Ontario, Canada).

Statistical Analysis

All values were expressed as mean ± SEM. For the analysis of regional OTR binding, two-way ANOVA was performed for factors ‘treatment (saline/nicotine)’ and ‘brain region’ at different bregma levels. Bonferroni *post-hoc* test was used when ANOVA reached significance (i.e., $p<0.05$). All statistical analyses were performed using *Statistica 8.0* (Statsoft Inc., France).

Results

High levels of OTR binding (0.97-1.15 fmol/mg tissue) were observed within the olfactory nuclei, medium binding levels (0.28-0.68 fmol/mg tissue) were identified within the mediolateral septum, ventral limb of the diagonal band of Broca, amygdala and hypothalamus, while low levels of binding (0.07-0.17 fmol/mg tissue) were observed in striatal regions (i.e., nucleus accumbens, caudate putamen and olfactory tubercle) as well as the thalamus (Figure 1A-E).

Olfactory nuclei: Two-way ANOVA showed a significant effect of 'brain region' ($F_{[2,24]} = 7.37$, $p < 0.01$), but no 'treatment' ($F_{[1,24]} = 0.30$, $p > 0.05$) or 'treatment' x 'brain region' interaction effect ($F_{[2,24]} = 0.01$, $p > 0.05$).

Striatum: Two-way ANOVA revealed a significant effect of 'brain region' ($F_{[2,24]} = 4.48$, $p < 0.05$), but no 'treatment' ($F_{[1,24]} = 0.26$, $p > 0.05$) or 'treatment' x 'brain region' interaction effect ($F_{[2,24]} = 0.06$, $p > 0.05$).

Septum: Two-way ANOVA revealed a significant effect of 'brain region' ($F_{[2,24]} = 10.06$, $p < 0.001$), but no 'treatment' ($F_{[1,24]} = 0.64$, $p > 0.05$) or 'treatment' x 'brain region' interaction effect ($F_{[2,24]} = 0.09$, $p > 0.05$).

Forebrain: Two-way ANOVA revealed a significant effect of 'brain region' ($F_{[3,27]} = 53.50$, $p < 0.001$) and 'treatment' x 'brain region' interaction effect ($F_{[3,27]} = 3.40$, $p < 0.05$). Bonferroni's *post-hoc* comparison test showed a significant, 46% increase of OTR binding in the amygdala following nicotine treatment (*$p<0.01$).* No effects of nicotine administration on the OTR binding were observed in the hippocampus, thalamus, or hypothalamus *($p>0.05$).*

Discussion

The present study demonstrated, for the first time, a region-specific alteration of the OTR binding in the brain of mice treated with a chronic nicotine administration paradigm. This up-regulation of the OTR was specifically localized in the amygdala, a region involved in stress and emotional regulation [27,28]. Therefore, this oxytocinergic system alteration may be involved in the modulation of long-term behavioural adaptations induced by chronic nicotine exposure.

Expression and distribution of the OTR observed in the current study is in line with previous published data in mice [e.g. 20,29]. Although alterations of the endogenous oxytocin system have been previously demonstrated following acute or chronic alcohol [30], cocaine [18,19,31], methamphetamine [20], 3,4-methylenedioxymethamphetamine (MDMA) [32] and morphine [21,33,34] administration in the brain of rodents, this is the first study to report central oxytocinergic neuroadaptations following chronic nicotine administration. The up-regulation of OTR binding in the amygdala observed in the present study, is in line with studies investigating the effects of chronic opioid, cocaine and methamphetamine administration on the central oxytocinergic system [19,20,35], indicating a possible common mechanism of action of several drugs of abuse through the modulation of the amygdalar OTR.

The role of this nicotine-induced increase in OTR binding is yet to be determined. However, given the involvement of the amygdala in a number of facets of emotional regulation [27] and social cognition [36], we can postulate that the alterations observed in this study might reflect neuroadaptations of the OT system in response to emotional and cognitive impairments induced by chronic nicotine. Indeed, chronic nicotine administration has been shown to induce emotional deficits, including depression and anxiety [10]. This hypothesis is further supported by findings

demonstrating that the anxiolytic, antidepressant and stress-relieving properties of OT to be at least partly modulated by the amygdala [37].

Nicotine administration has been also shown to exert cognition-enhancing effects [38]. Since oxytocin is also considered as a key mediator of social cognition in humans [39] and this effect has been postulated to specifically involve the OT system in the Amy [40], the OTR up-regulation observed here might also be associated with a possible nicotine regulation of cognition through an OTR-dependent mechanism. However, this hypothesis needs to be further investigated.

Importantly, alterations of OTR in the amygdala have been previously associated with a hypo-oxytocinergic tone in the brain. In particular, chronic morphine administration and withdrawal induced a marked decrease in the hypothalamic OT levels, concomitant with a rebound increase of the OTR in the amygdala [21]. Considering these findings and since an increase in OT peptide levels or administration of an OTR agonist induces rapid OTR desensitization, clathrin-dependent internalisation and subsequent downregulation of the receptors [41], we can speculate that the up-regulation of OTR in the amygdala is a rebound consequence of a possible reduction of OT levels in the brain following chronic nicotine administration. Indeed, there is evidence for a reduced oxytocinergic tone following nicotine administration, as acute nicotine was shown to diminish OT levels in the pituitary of rats [24].

In conclusion, this is the first study to demonstrate direct brain region-specific alterations in the amygdalar OTR following nicotine administration in mice. This study suggests the alterations of OTR as one possible mechanism underlying behavioural and neurochemical alterations observed in nicotine addiction.

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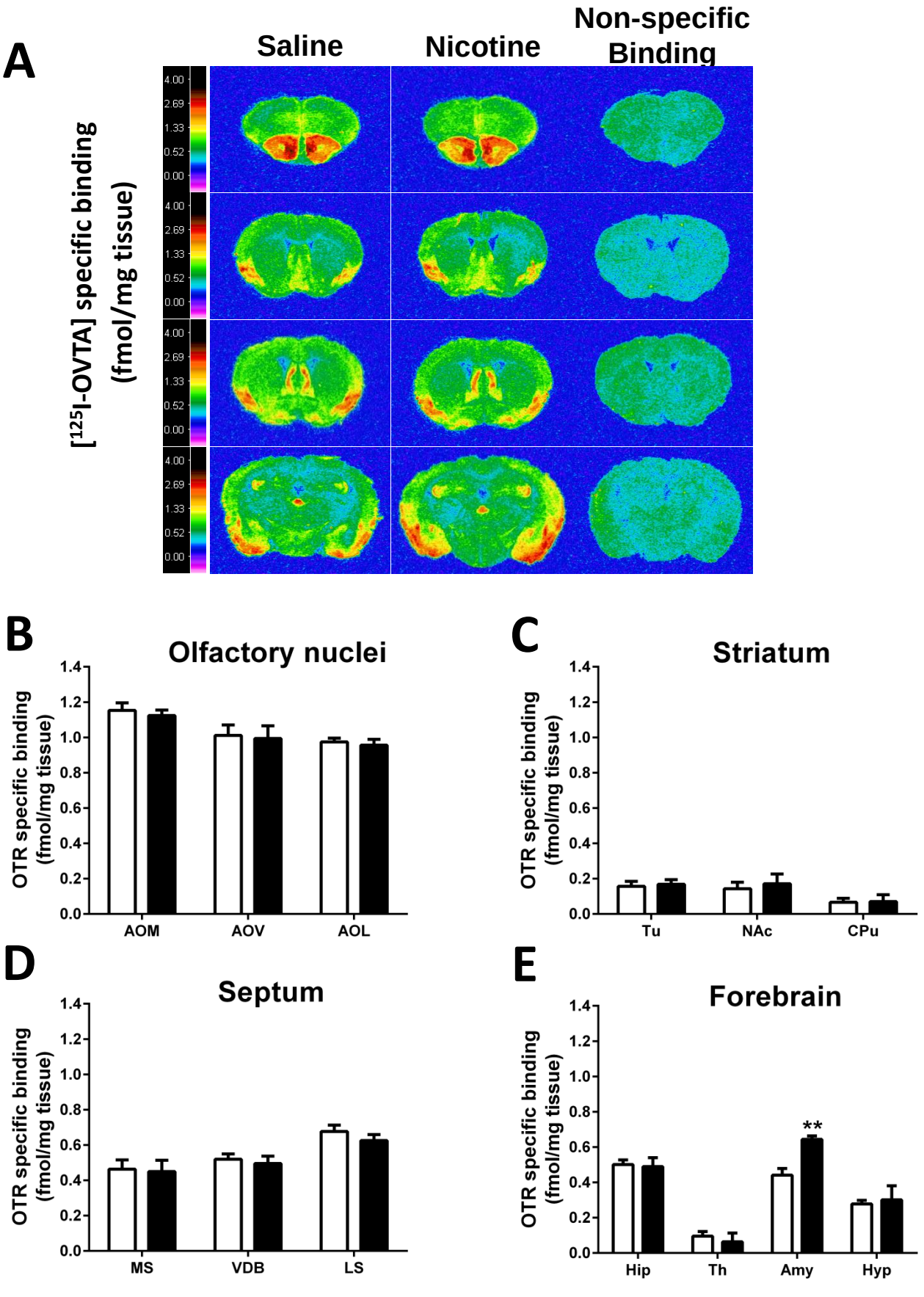
Figure Legend

Figure 1: ***Oxytocin receptor (OTR) binding in the brain of mice following long-term nicotine administration*****.** C57BL/6J mice were treated for fourteen days with either saline or nicotine via osmotic minipumps. **(A)** Representative autoradiograms of 50 pM [^{125}I]-ornithine vasotocin analogue binding to OTR in coronal brain sections at the level of the olfactory nuclei (row 1), striatum (row 2), septum (row 3) and forebrain (row 4). Binding levels are represented using a pseudo-colour interpretation of black and white film images in fmol/mg of tissue equivalent. Quantitative OTR binding levels at the level of **(B)** olfactory nuclei, **(C)** striatum**, (D)** septum and **(E)** forebrain, where OTRs are expressed. Data are expressed as mean ± SEM (n=4-6 per treatment group). $^{**}p<0.01$ *vs* saline control. Abbreviations: Acb, nucleus accumbens; Amy, amygdala; AOL, anterior olfactory nucleus-lateral; AOM, anterior olfactory nucleus-medial; AOV, anterior olfactory nucleus-ventral; CPu, caudate putamen; Hip, hippocampus; Hyp, hypothalamus; LS, lateral septum; MS, medial septum; Th, thalamus; Tu, olfactory tubercle; VDB, vertical limb of diagonal band of Broca.


A
Saline
Nicotine
Non-specific Binding
[125I-OVTA] specific binding (fmol/mg tissue)
4.00
2.69
1.33
0.52
0.00
B
Olfactory nuclei
OTR specific binding (fmol/mg tissue)
AOM
AOV
AOL
C
Striatum
Tu
NAc
CPu
D
Septum
MS
VDB
LS
E
Forebrain
Hip
Th
Amy
Hyp
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