TITLE PAGE

Chronic nicotine administration restores brain region specific up-regulation of oxytocin receptor binding levels in a G72 mouse model of schizophrenia

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ABSTRACT

Nicotine dependence and schizophrenia are two mental health disorders with remarkably high comorbidity. Cigarette smoking is particularly prevalent among schizophrenic patients and it is hypothesized to comprise a form of self-medication for relieving cognitive deficits in these patients. Emerging evidence suggests a role of the neurohypophysial peptide oxytocin in the modulation of drug addiction, as well as schizophrenia symptomology; however, the underlying mechanism remains unclear. Therefore, we sought to investigate the effects of chronic nicotine administration on oxytocin receptor (OTR) binding in the brain of a transgenic mouse model of schizophrenia that carries a bacterial artificial chromosome of the human G72/G30 locus (G72Tg). Female wild-type (WT) and heterozygous G72 transgenic CD-1 mice were treated with a chronic nicotine regimen (24 mg/kg/day, osmotic minipumps for 14 days) and quantitative autoradiographic mapping of oxytocin receptors was carried out in brains of these animals. OTR binding levels were higher in the cingulate cortex (CgCx), nucleus accumbens (Acb) and central amygdala (CeA) of saline treated G72Tg mice compared with WT control mice. Chronic nicotine administration reversed this upregulation in the CgCx and CeA. Interestingly, chronic nicotine administration induced an increase in OTR binding in the CeA of solely WT mice. These results indicate that nicotine administration normalizes the dysregulated central oxytocinergic system of this mouse model of schizophrenia and may contribute towards nicotine's ability to modulate cognitive deficits which are common symptoms of schizophrenia.

INTRODUCTION

Schizophrenia is a neuropsychiatric disorder, typically manifested during adolescence or early adulthood and is usually characterised by hallucinations, delusions, impaired cognitive function and emotional deficits (American Psychiatric Association, 2013). Impaired cognitive function in schizophrenia includes deficits in sensory gating (Adler *et al.*, 1998), smooth pursuit eye movement (Olincy *et al.*, 1998), working memory (Gold *et al.*, 2006; Nuechterlein *et al.*, 2008) and social cognition (Derntl & Habel, 2011).

Converging lines of evidence indicate the presence of high comorbidity rates between nicotine dependence and schizophrenia; smoking prevalence among schizophrenics is estimated at 74-92%, compared with 35-54% for all psychiatric patients and 20% for the general population (see Adler *et al.*, 1998; D'Souza & Markou, 2012). Nicotine has been shown to transiently ameliorate attention (Dépatie *et al.*, 2002; Harris *et al.*, 2004), working memory (Nuechterlein *et al.*, 2004) and sensory gating (Adler *et al.*, 1998; Olincy *et al.*, 1998) deficits in schizophrenic patients, indicating a possible use of nicotine via smoking as a self-medication in these patients (Glassman, 1993; Kumari & Postma, 2005). By using the G72Tg mouse model of schizophrenia, we found that chronic nicotine administration was effective in restoring impaired prepulse inhibition, working memory, associative learning and social recognition in G72Tg mice further supporting the beneficial attributes of nicotine in modulating cognitive deficits symptoms of schizophrenia (Hambsch *et al.*, 2014). Although the benefits of nicotine use in schizophrenia have been broadly studied, the neurobiology underlining this nicotine dependence-schizophrenia comorbidity remains unclear.

There is emerging evidence suggesting that the central oxytocinergic system plays a key role in the psychopathology of both drug addiction, including nicotine dependence, and schizophrenia. Research into the underlying mechanisms of cognitive deficits associated with schizophrenia has revealed a key role of the oxytocinergic system, primarily due to its

modulatory role in social cognition (Stoop, 2012). In particular, intranasal oxytocin (OT) treatment in schizophrenic patients abolishes psychotic symptoms (Bujanow, 1974), reduces positive and negative symptoms of schizophrenia (Feifel *et al.*, 2010) and improves social perception (Pedersen *et al.*, 2011). Similarly, OT administration restores social behavior which is profoundly impaired in animal models of schizophrenia (Lee *et al.*, 2007). Previous studies have also identified reduced plasma OT levels in schizophrenic patients (Walss-Bass *et al.*, 2013), suggesting a possible dysregulation of the oxytocinergic system in some schizophrenia-related symptomology. Indeed, higher OT levels in the blood were directly correlated with reduced schizophrenia-related symptoms in women (Sasayama *et al.*, 2012). Several OT receptor (OTR) single nucleotide polymorphisms (SNPs) have been associated with increased severity of various schizophrenia-like symptoms. In particular, rs2254298, rs53576 or rs237885 SNPs on the *otr* gene have all been associated with severe psychopathology and negative symptoms of schizophrenia (Montag *et al.*, 2012). Overall, these studies provide evidence that the oxytocinergic system is likely to be involved in the underlying psychopathology of schizophrenia.

In addition to schizophrenia, the OT system has been also widely implicated in drug addiction processes. Specifically, chronic administration of drugs of abuse such as cocaine (Sarnyai *et al.*, 1992; Georgiou *et al.*, 2015a), methamphetamine (Zanos *et al.*, 2014b; Georgiou *et al.*, 2016), morphine (Zanos *et al.*, 2014a), alcohol (Silva *et al.*, 2002) and nicotine (Zanos *et al.*, 2015) induce alterations in the central OTR system. Moreover, prenatal combined treatment with alcohol and nicotine decreased OT levels (McMurray *et al.*, 2008) and increased OTR binding in the brain of rats (Williams *et al.*, 2009). Acute intravenous administration of nicotine has been shown to decrease OT content in the pituitary of rats (Russell & Chaudhury, 1972), and systemic administration of OT abolished physical somatic symptoms of nicotine withdrawal in rat (Manbeck *et al.*, 2014) and reduced cigarette consumption in daily smokers

(Van Hedger *et al.*, 2018). There is ample evidence clearly demonstrating that OT administration can modulate addiction related behaviors (McGregor & Bowen, 2012; Zanos *et al.*, 2017) and can prevent relapse (Zanos *et al.*, 2014a; Georgiou *et al.*, 2015b) and thus has been proposed as a novel treatment for drug addiction. Interestingly, due to the anxiolytic, antidepressant and prosocial properties of OT, there is particular interest in its potential efficacy in the treatment of mental health disorder comorbidities including addiction-depression comorbidity (Zanos *et al.*, 2017). Nonetheless, the role of OT in addiction-schizophrenia comorbidity remains unclear.

Genome-wide association studies have implicated the *G72/G30* gene locus (13q32-q34) in susceptibility to schizophrenia (for review see Drews *et al.*, 2012). *G72* is a primate-specific gene encoding the G72 protein in humans, and a truncated form in non-human primates. The G72 protein deactivates D-amino acid oxidase (DAOA) (Sacchi *et al.*, 2008) thus preventing the oxidation of D-serine, an activator of the NMDA glutamate receptor (Chumakov *et al.*, 2002) by decreasing the glutamatergic signalling. Abnormalities in the glutamatergic system, particularly in the cortical regions of the brain, have been implicated in the pathophysiology of schizophrenia (for reviews see Olney & Farber, 1995; Adell *et al.*, 2012), and transgenic mice with decreased NMDA receptor levels display schizophrenia-like behavioral abnormalities (Mohn *et al.*, 1999).

To investigate the possible involvement of OT in the mechanism underlining nicotine dependence and schizophrenia comorbidity, we aimed to assess if OTR levels were dysregulated in the brains of the G72Tg mouse model of schizophrenia and whether chronic nicotine treatment was able to modulate such dysregulation.

METHODS

Animals and chronic nicotine administration paradigm

WT and heterozygous G72Tg transgenic CD-1 mice were originally generated by Otte et. al., (2009), at the University of Bonn. The animals were individually housed, 12:12 h reversed light/dark-cycle, in a temperature-controlled environment with food and water available *ad libidum*. Female mice (16 G72Tg and 17 WT littermates, total 33 animals, **10-12 weeks old, 31-35 grams**) were administered with chronic saline (8 G72Tg, 10 WT) or (-)-Nicotine hydrogen tartrate salt (24 mg/kg/day; Sigma, St. Louis, MO, USA) delivered in physiological saline (0.9%) (8 G72Tg and 7 WT) for 14 days using Alzet® osmotic minipumps (Charles River GMbH, Kißlegg, Germany). This treatment protocol is known to produce nicotine plasma concentrations similar to those found in human smokers (Hambsch *et al.*, 2014). For minipump implantation, mice were anaesthetised using isoflurane/oxygen vapor mixture (3.5%–4.5%; Isoflo, Abbott Laboratories Ltd, UK). 0.2 mg/kg meloxicam (i.m). was used for analgesia. 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. Since the behavioral effects of nicotine were more pronounced in female compared with male mice in this transgenic line (Hambsch *et al.*, 2014) autoradiography was undertaken only in the brains of female mice. Animal work was carried out in accordance with German and EU regulations (European Communities Council Directive 2010/63EU) and was approved by local authorities (University of Bonn animal experimentation ethics committee file-no.87-51.04.2010.A070). The animal work was reported according to ARRIVE guidelines. For checklist assessing compliance to ARRIVE guidelines please see Supplementary Table. 1.

Quantitative receptor autoradiography

Following 14 days of chronic nicotine treatment, mice were killed by cervical dislocation, brains were removed and frozen using isopentane solution (-20^{o}C) for 30 sec and stored at -80 ^{o}C, until use. Adjacent 20 μm coronal brain sections were cut at 300 μm intervals from fore to hind brain (complete mapping) at -21 °C using a cryostat apparatus and thaw mounted onto ice-cold microscope slides. Quantitative autoradiography was performed on brain sections from chronic saline- and nicotine-treated WT and G72Tg mice in accordance with previously described methods (Jarrett *et al.*, 2006; Zanos *et al.*, 2014b). 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) as previously described by Kitchen et al. (1997). Brain structures were identified by reference to the mouse atlas of Franklin and Paxinos (2001).

Statistical analysis

Data are presented as mean ± SEM and were analysed using GraphPad Prizm software. The same regions were analysed in all mice but binding in some regions could not be analysed due to either damage to the brains or sections thus the sample size change between brain regions. For the determination of differences in OTR binding between the different treatment

groups, two-way ANOVA for each brain region were performed with factors 'treatment' and 'genotype', followed by Holm-Sidak *post-hoc* test. Statistical significance was set to $p \leq 0.05$.

RESULTS

Effect of nicotine on oxytocin binding in WT and G72Tg mice

High levels of OTR binding (2.85–4.99 fmol/mg tissue) were found in the olfactory nuclei, piriform/endopiriform cortices, lateral septum and amygdala; medium binding levels (0.96–2.54 fmol/mg tissue) in the cingulate cortex, olfactory tubercle, medial septum, ventral limb of the diagonal band of Broca and hypothalamus; and low levels (0.24–0.57 fmol/mg tissue) in the striatal areas (i.e., nucleus accumbens and caudate putamen), as well as the thalamus (Figures 1 and 2).

A significant treatment effect was observed in the medial septum ($F_{(1, 24)} = 4.01$; $p = 0.05$) (Table 1; Fig. 2). Holm-Sidak *post-hoc* analysis showed a near significant increase in OTR binding in saline-treated G72Tg compared with the saline-treated WT mice ($p = 0.09$), which returned to control levels following chronic nicotine administration (saline treated G72Tg *vs* nicotine treated G72Tg; $p < 0.05$; n = 6-8/group). In the nucleus accumbens, two way ANOVA revealed a significant genotype effect ($F_{(1, 24)} = 4.01$; $p = 0.05$) but no treatment or treatment x genotype interaction effects ($p > 0.05$).

A significant genotype x treatment interaction effect was detected in the cingulate cortex ($F_{(1, 23)} = 4.86$; $p = 0.04$) and the central amygdala ($F_{(1, 24)} = 15.05$; $p = 0.0007$) with a near significant interaction effect observed in the anterior olfactory nucleus (medial) ($F_{(1, 19)} = 3.66$; $p = 0.07$). No individual treatment or genotype effects were observed in these regions ($p > 0.05$). Holm-Sidak *post-hoc* analysis showed near significantly higher levels of OTR in the anterior olfactory nucleus (medial) ($p = 0.08$) and significantly higher levels of OTR in the cingulate cortex ($p < 0.05$) and the central amygdala ($p < 0.05$) of saline treated G72Tg mice compared with WT.

Chronic nicotine treatment restored OTR levels back to control WT saline levels in G72Tg mice in all these regions ($p<0.05$, saline-treated G72Tg compared with nicotine-treated G72Tg mice; $p>0.05$, saline WT compared to nicotine G72Tg). Interestingly, chronic administration of nicotine increased OTR binding only in the central amygdala of WT compared with saline WT controls ($p<0.01$; n = 6–8/group). This nicotine induced upregulation was not observed in G72Tg mice ($p>0.05$).

No other genotype, treatment or genotype x treatment interaction effects were observed in any other regions analysed ($p>0.05$).

DISCUSSION

In the present study, we found increased OTR binding in the cingulate cortex and central amygdaloid nucleus in the brain of a G72Tg mouse model of schizophrenia compared with WT; this effect was reversed by chronic nicotine administration. In addition, we showed an effect of chronic nicotine administration in increasing OTR binding in the central amygdala of WT mice in line with our previous findings (Zanos *et al.*, 2015). This nicotine-induced upregulation was absent in G72Tg mice.

Higher rates of smoking among schizophrenics exist compared with the general population and schizophrenic patients tend to be heavier smokers, obtaining higher levels of nicotine per cigarette (de Leon & Diaz, 2005). We have previously shown, using the same administration paradigm, that chronic nicotine treatment was able to reverse several cognitive deficits in the G72Tg mouse model of schizophrenia (Hambsch *et al.*, 2014) supporting the concept that schizophrenic patients may use nicotine from cigarettes to self-medicate some of the symptoms of schizophrenia (Glassman, 1993; Kumari & Postma, 2005). More specifically, we showed that chronic nicotine administration was effective in restoring impaired prepulse inhibition, working memory, associative learning and social recognition in G72Tg mice, further supporting the beneficial attributes of nicotine in modulating cognitive deficits

symptoms of schizophrenia (Hambsch *et al.*, 2014). However, the mechanisms underlying the beneficial effects of nicotine treatment for alleviating negative symptoms of schizophrenia, including impaired cognition, are still not well understood. Since oxytocin has been implicated in the different aspects of cognition (Lee *et al.*, 2015; Crespi, 2016) and social information processing (Freeman *et al.*, 2014a; Freeman *et al.*, 2014b), both of which are disrupted in schizophrenic patients (Bowie & Harvey, 2006), we assessed the regulation of the oxytocinergic system in the brains of the G72Tg mouse model of schizophrenia.

We decided to focus this study on female G72Tg mice based on our previous observations indicating that the restorative effect of nicotine on pre-pulse inhibition deficits and certain cognitive deficits in G72Tg mice are more pronounced in female mice compared with male mice (Hambsch *et al.*, 2014). This is consistent with the gender differences observed in characteristics and behaviours of smokers diagnosed with psychosis (Filia *et al.*, 2014). Thus, in order to elucidate corresponding molecular mechanisms underlying the beneficial effects of nicotine in G72Tg mice, we focused our OT receptor autoradiographic study on female WT and G72Tg mice.

Here we demonstrate a dysregulation of OTR density in G72Tg mice in brain regions associated with social cognition and social processing. Indeed, the upregulation of OTR observed in the G72Tg mouse model of schizophrenia were localized in the cingulate cortex and amygdala, regions that have been shown to mediate the effects of OT on social cognition and social processing (Zink & Meyer-Lindenberg, 2012). In addition, a neuronal interaction between the cingulate cortex and amygdala has been shown to mediate fear processing (Williams *et al.*, 2006), which is also impaired in schizophrenics (Michalopoulou *et al.*, 2008), suggesting that oxytocinergic dysregulation within these brain circuits might underlie some of the negative symptoms of schizophrenia. Increases in OTR binding were also observed in the medial septum and nucleus accumbens of the G72Tg mouse model of schizophrenia in the

present study but this upregulation did not quite reach statistical significance. Notably, deep brain stimulation specifically in these two brain regions was shown to alleviate psychotic symptoms in rats (Ma & Leung, 2014), suggesting that changes in the OTR system in both these brain areas might be involved in psychosis observed in schizophrenic patients; however the exact role of OT in the modulation of such behaviors requires further investigation.

Although the molecular mechanism underpinning the upregulation of OTR in certain brain regions of G72Tg mice cannot be determined from this study, it is likely that this may be driven by alterations in central OT levels. An increase in OT peptide or repeated activation of OTRs is known to cause a rapid desensitisation of OTRs and consequently decreases receptor binding in cells (Evans *et al.*, 1997) and brains of animals (Peters *et al.*, 2014; Rae *et al.*, 2018) Rae et al., 2018; Peters et al.,). Several studies have reported low levels of central OT go hand in hand with high OTR density in the brain of the same animals (Lee *et al.*, 2007; Zanos *et al.*, 2014a). Interestingly, this central oxytocinergic dysregulation has been shown to be concomitant with the emergence of social deficit and emotional impairment, behaviors which were reversed by administration of the OT or OT analogues (Zanos et al. 2014; (Green & Leitman, 2008), pointing towards a causal relationship between central oxytocinergic dysregulation and socio-emotional impairment. Therefore, we can postulate that the increased OTR binding observed in the present study is caused by a reduction in OT peptide levels in the brain as a compensatory neuroadaptive mechanism. This is in line with decreased plasma OT levels and hypothalamic OT mRNA levels found in schizophrenic patients (Walss-Bass *et al.*, 2013) and rodents (Lee *et al.*, 2007) respectively, an effect which was concomitant with an increase in OTR binding in the central nucleus of the amygdala of a different rodent model of schizophrenia (Lee *et al.*, 2007) Moreover, several studies have demonstrated beneficial effects of OT in treating social cognitive deficits in schizophrenic patients (Feifel & Shilling, 2010; Fischer-Shofty *et al.*, 2013; see also Miyamoto *et al.*, 2013a), further supporting the hypothesis

that dysregulated central oxytocinergic system may mediate some of the symptoms observed in schizophrenia.

Interestingly, here we show that chronic nicotine treatment reverses the brain specific upregulated OTR observed in G72Tg mice clearly indicating that the dysregulated central OTR system is under the regulatory control of nicotine. Whether this regulatory effect of nicotine on the OTR system of G72Tg mice underlines the beneficial effect of nicotine on alleviating cognitive deficits experienced by schizophrenic patients cannot be determined from this study. Nonetheless the fact that these nicotine-induced oxytocinergic neuroadaptations are concomitant with a complete nicotine induced reversal of impaired prepulse inhibition, working memory, associative learning and social recognition detected in G72Tg mice (Hambsch *et al.*, 2014) makes this hypothesis plausible. Interestingly, the effects of nicotine on attention performance (Mobascher *et al.*, 2012) and working memory (Ernst *et al.*, 2001) have been associated with an activation of the anterior cingulate cortex, supporting our hypothesis of an involvement of the OTR system in the cingulate cortex to underlie the beneficial effects of nicotine on schizophrenia. Moreover, we have demonstrated that chronic nicotine administration abolished increases in OTR binding within the medial septum of G72Tg mice, a region associated with social memory (Lukas *et al.*, 2013). Given the key role of the septum in mediating the beneficial effects of nicotine on social cognition, which is impaired in schizophrenics (Beck *et al.*, 2015), together with oxytocins' cognitive enhancement properties (Zink & Meyer-Lindenberg, 2012), it would be intriguing to suggest a possible role of the septal OT system in modulating the enhancement of social cognition by chronic nicotine administration in mouse model of schizophrenia.

Although [^{125}I]-ornithine vasotocin (OVTA) used to label OTR in our study is highly selective antagonist for oxytocin receptors and has negligible affinity for vasopressin receptors (Elands *et al.*, 1988), one cannot entirely exclude the possibility that OVTA binding may reveal

a small proportion of vasopressin receptors in addition to OTRs. In addition, due to the structural similarity of OT to the related neuropeptide vasopressin, and hence the cross-reactivity of the latter to OTRs (Neumann & Landgraf, 2012), we cannot rule out the possibility that nicotine-induced increase in vasopressin release could also contribute to the normalization of the expression of OTRs in some of the brain regions of G72Tg mice. Indeed, there is some evidence that nicotine can also induce vasopressin secretion from the hypothalamus (Fuxe *et al.*, 1983).

Using the same G72Tg mouse model of schizophrenia, we have previously shown that chronic nicotine administration reversed increases in α7 nicotinic receptor binding within the cingulate cortex of G72Tg compared with WT mice. This is particularly important since adjunctive treatment with α7 nAChR agonists and OT is believed to be promising in the treatment of negative symptoms of schizophrenia, including psychotic symptoms and cognitive deficits (Miyamoto *et al.*, 2013b). Therefore, an interaction between the oxytocinergic and nicotinic acetylcholine receptor systems might exist in the brain to mediate several of the negative symptoms of schizophrenia. This hypothesis warrants further investigation, especially in the light of the findings of Zaninetti et al., (2002), who showed that nicotinic agonists can directly increase excitability of OT magnocellular neurons. Additionally, nicotine administration was shown to directly induce a marked activation (85%) of the oxytocinergic neurons in the hypothalamus of rats (Mikkelsen *et al.*, 2012), where α7 nicotinic acetylcholine receptors were shown to be expressed (Hatton & Yang, 2002), further supporting an interaction between these systems.

Altogether, the current findings suggest the possibility that nicotine treatment improves at least some of the symptoms of schizophrenia via an oxytocinergic mechanism, thus supporting the self-medication hypothesis, that schizophrenic patients might smoke more than non-schizophrenic populations in an attempt to self-medicate distressing cognitive symptoms

(Kumari & Postma, 2005). While smoking is known to be harmful to health (The Health and Social Care Information Centre, 2012) and current guidelines recommend a variety of smoking cessation methods for people suffering from schizophrenia (NICE, 2015), traditional NRT and/or novel nicotine containing products such as electronic cigarettes may be useful not only for improving smoking cessation rates (Hickling *et al.*, 2018), but also to treat some of the symptoms of schizophrenia. Interestingly, the beneficial effects of nicotine on cognitive behavior in G72Tg mice were observed following relatively high doses (24 mg kg^{-1} day^{-1}, also used in the present study)-albeit within the range observed in smokers- but not at low dose (16 mg kg^{-1} day^{-1}) of nicotine exposure (Hambsch *et al.*, 2014), which is in line with the higher cigarette consumption and nicotine intake rate of schizophrenic patients compared with control smokers (Olincy *et al.*, 1997) It is therefore possible that higher levels of nicotine are required to normalise the upregulated OTRs in G72Tg mice brains and hence alleviate some of the cognitive deficits in these animals, and may have implications on the dose of nicotine recommended for schizophrenic smokers undergoing nicotine containing smoking cessation methods. As there are safety concerns regarding very high doses of nicotine, a useful alternative may consist of an adjunctive treatment of OT which is known to also known to cause downregulation of OTRs in the brain (Peters *et al.*, 2014) with "safer" doses of nicotine. Such targeted pharmacotherapies in this specific population warrants further investigation.

In conclusion, the present study demonstrates that dysregulated OTRs in a mouse model of schizophrenia are under regulatory control of nicotine, suggesting a possible interaction between nAChR and OTR systems in the mechanism underlying nicotine dependence-schizophrenia comorbidity. Whether there is a causal relationship between this regulatory control of nicotine and its beneficial effect in ameliorating cognitive symptoms of schizophrenia warrants further investigation.

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CONFLICT OF INTEREST STATEMENT

The sponsors had no involvement in the design of the study and in the collection, analyses and interpretation of the data, nor in the writing of the report and the decision to submit this article for publication. The authors report no conflict of interest and no biomedical financial interest from this research.

AUTHOR CONTRIBUTIONS

H. K., P.Z., P.G., I.K., A.Z., A.B. conceived and designed the experiments. H. K., and P.Z. performed the experiments. H. K., P.G., P.Z. and A. B. analysed the data. A.Z. and A.B. contributed reagents/materials/analysis tools. H. K., P.Z., P.G., I.K., A.Z., A.B. wrote or commented on the manuscript.

DATA ACCEPTABILITY

Data will be available upon request from the corresponding author.

ABREVIATIONS

ornithine vasotocin (OVTA); non-specific binding (NSB); cingulate cortex (CgCx), nucleus accumbens (Acb), medial septum (MS) and central amygdala (CeA); wild type (WT);

transgenic mice carries a bacterial artificial chromosome of the human G72/G30 locus (G72Tg); oxytocin (OT); oxytocin receptors (OTR)

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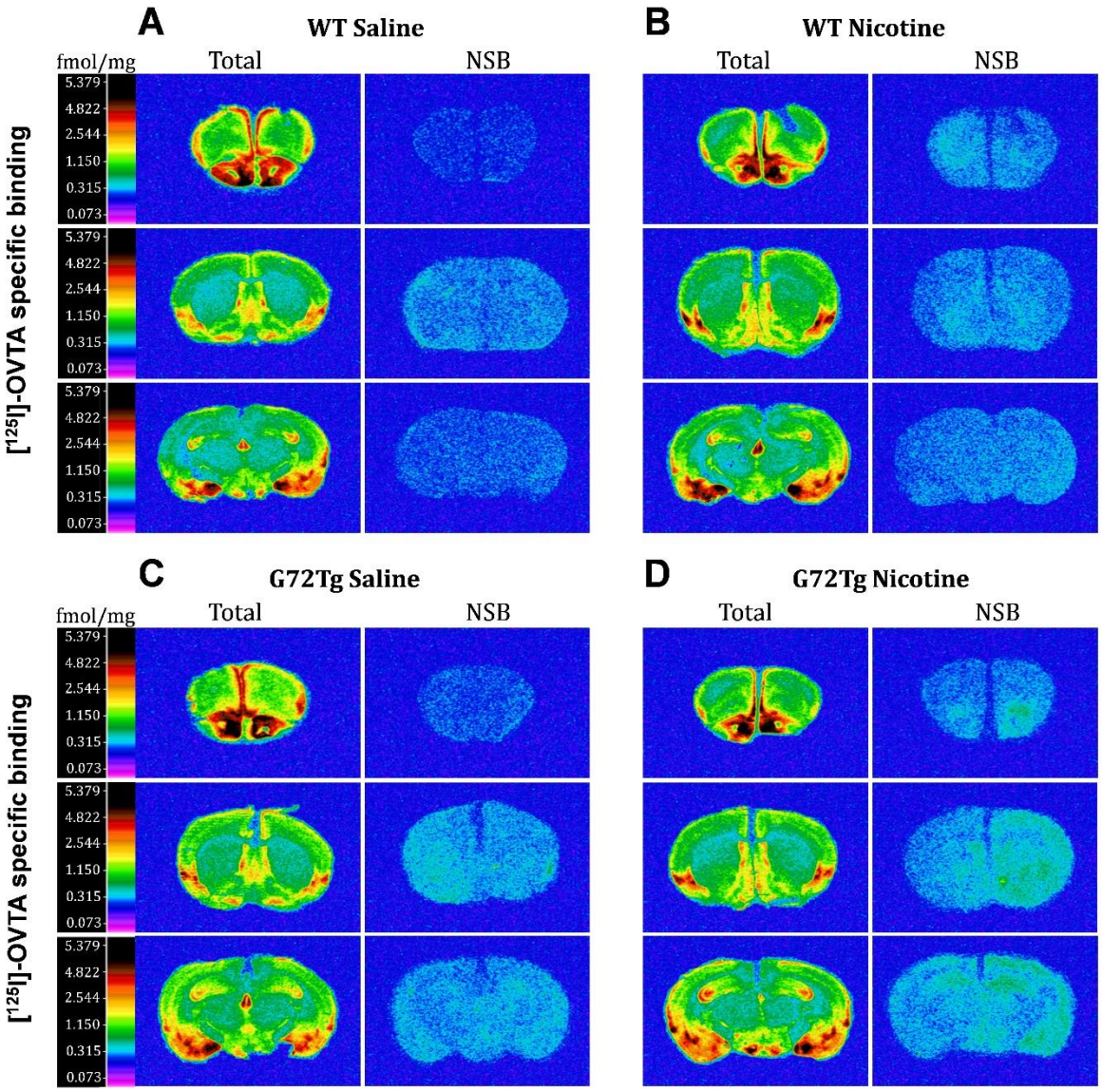
Table 1. Two Way ANOVA factorial results

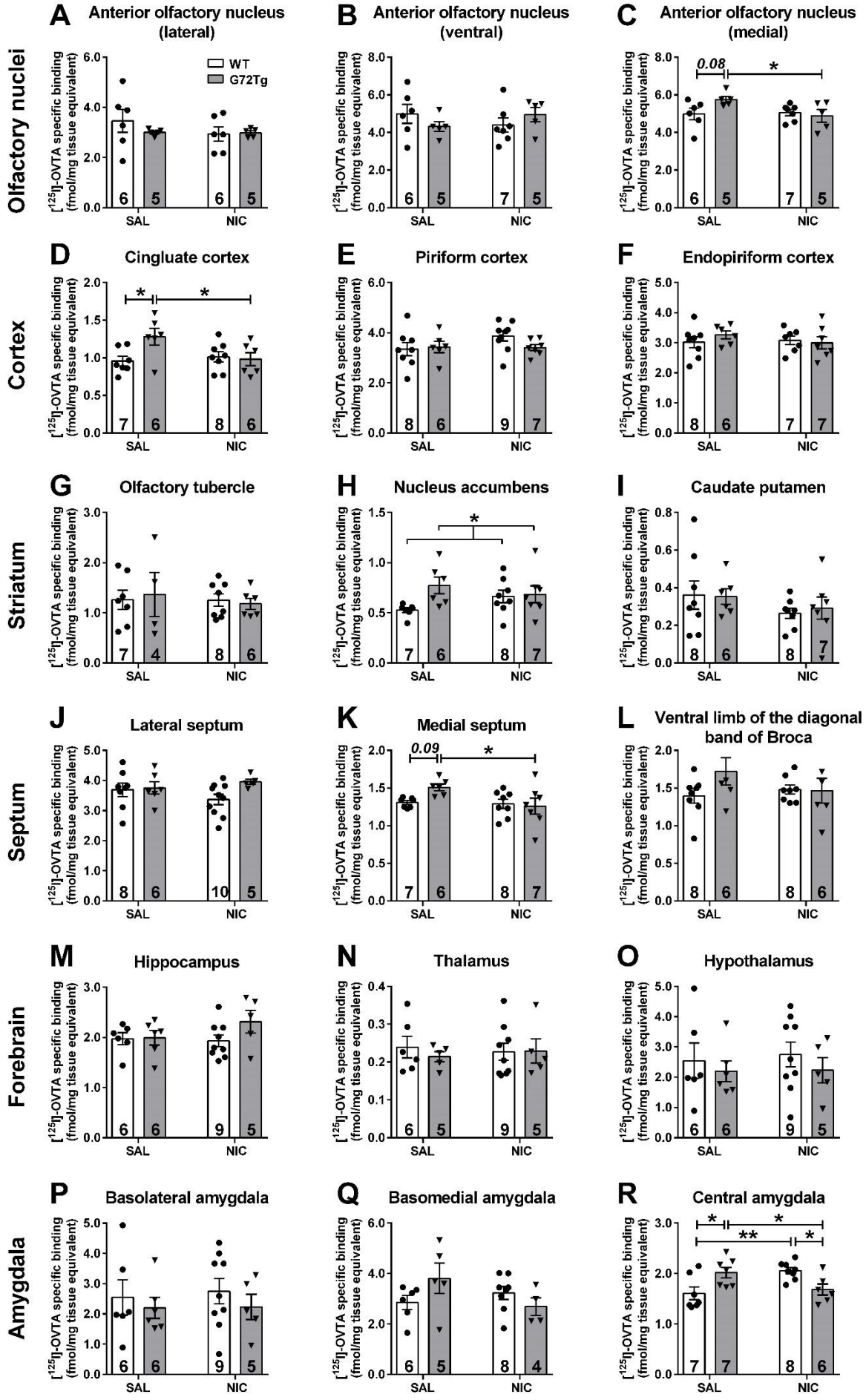
Brain region	Treatment	Genotype	Interaction	Animal numbers
Anterior olfactory nucleus-lateral	$F_{(1, 18)} = 0.82$; $p = 0.38$	$F_{(1, 18)} = 0.47$; $p = 0.50$	$F_{(1, 18)} = 0.71$; $p = 0.41$	n = 5-7
Anterior olfactory nucleus-ventral	$F_{(1, 19)} = 0.002$; $p = 0.96$	$F_{(1, 19)} = 0.02$; $p = 0.89$	$F_{(1, 19)} = 2.28$; $p = 0.15$	n = 5-6
Anterior olfactory nucleus-mediall	$F_{(1, 19)} = 2.54$; $p = 0.13$	$F_{(1, 19)} = 1.44$; $p = 0.25$	$F_{(1, 19)} = 3.66$; $p = 0.07$	n = 5-7
Cingulate cortex	$F_{(1, 23)} = 2.23$; $p = 0.15$	$F_{(1, 23)} = 3.28$; $p = 0.08$	$F_{(1, 23)} = 4.86$; $p = 0.04$ * [% 14.88]	n = 6-8
Piriform cortex	$F_{(1, 26)} = 1.35$; $p = 0.26$	$F_{(1, 26)} = 0.78$; $p = 0.39$	$F_{(1, 26)} = 1.65$; $p = 0.21$	n = 6-9
Endopiriform cortex	$F_{(1, 24)} = 0.33$; $p = 0.57$	$F_{(1, 24)} = 0.21$; $p = 0.65$	$F_{(1, 24)} = 0.88$; $p = 0.36$	n = 6-8
Olfactory tubercle	$F_{(1, 21)} = 0.23$; $p = 0.64$	$F_{(1, 21)} = 0.007$; $p = 0.93$	$F_{(1, 21)} = 0.19$; $p = 0.66$	n = 4-8
Nucleus accumbens	$F_{(1, 24)} = 0.13$; $p = 0.72$	$F_{(1, 24)} = 4.01$; $p = 0.05$ * [13.08%]	$F_{(1, 24)} = 2.28$; $p = 0.11$	n = 6-8
Caudate putamen	$F_{(1, 25)} = 2.00$; $p = 0.17$	$F_{(1, 25)} = 0.02$; $p = 0.88$	$F_{(1, 25)} = 0.10$; $p = 0.76$	n = 6-8
Lateral septum	$F_{(1, 25)} = 0.09$; $p = 0.77$	$F_{(1, 25)} = 2.57$; $p = 0.12$	$F_{(1, 25)} = 1.72$; $p = 0.20$	n = 5-10
Medial septum	$F_{(1, 23)} = 4.01$; $p = 0.05$ * [12.58%]	$F_{(1, 24)} = 1.69$; $p = 0.21$	$F_{(1, 24)} = 3.00$; $p = 0.09$	n = 6-8
Ventral limb of the diagonal band of Broca	$F_{(1, 24)} = 0.48$; $p = 0.50$	$F_{(1, 24)} = 1.66$; $p = 0.21$	$F_{(1, 24)} = 1.91$; $p = 0.18$	n = 6-8
Hippocampus	$F_{(1, 22)} = 0.90$; $p = 0.35$	$F_{(1, 22)} = 1.76$; $p = 0.20$	$F_{(1, 22)} = 1.48$; $p = 0.24$	n = 5-9
Thalamus	$F_{(1, 21)} = 0.003$; $p = 0.96$	$F_{(1, 21)} = 0.20$; $p = 0.66$	$F_{(1, 21)} = 0.27$; $p = 0.61$	n = 5-9
Hypothalamus	$F_{(1, 22)} = 0.07$; $p = 0.80$	$F_{(1, 22)} = 0.86$; $p = 0.36$	$F_{(1, 22)} = 0.03$; $p = 0.85$	n = 5-9
Basolateral amygdala	$F_{(1, 21)} = 0.02$; $p = 0.88$	$F_{(1, 21)} = 0.24$; $p = 0.63$	$F_{(1, 21)} = 0.15$; $p = 0.70$	n = 4-9
Basomedial amygdala	$F_{(1, 19)} = 0.93$; $p = 0.35$	$F_{(1, 19)} = 0.31$; $p = 0.58$	$F_{(1, 19)} = 3.84$; $p = 0.07$	n = 4-8
Central amygdala	$F_{(1, 24)} = 0.28$; $p = 0.60$	$F_{(1, 24)} = 0.03$; $p = 0.87$	$F_{(1, 24)} = 15.05$; $p = 0.0007$ *** [37.88%]	n = 6-8
*p ≤0.05; ***p ≤0.001				
% of total variation (eta squared) is included in brackets				

FIGURE LEGENDS

Figure 1: ***Representative autoradiograms of [^{125}I]OVTA binding following chronic saline or nicotine treatment in wild type (WT) and G72 transgenic (G72Tg) mice***. Computer enhanced colour autoradiograms of 50 pmol [^{125}I]-ornithine vasotocin analogue ([^{125}I]OVTA) binding to oxytocin receptors (OTR) in coronal brain sections of WT and G72Tg mice treated with chronic saline or nicotine (24 mg/kg/day) via subcutaneous minipumps for 14 days. Coronal brain sections are represented at the level of the olfactory nuclei (row 1; Bregma 2.46 mm), striatum (row 2; Bregma mm), septum (row 3; Bregma 0.86 mm). Adjacent sections were incubated with 50 μM oxytocin to determine non-specific binding (NSB). Binding levels are represented using a pseudo-color interpretation of black and white film images in fmol/mg of tissue equivalent.

Figure 2: ***Quantitative [^{125}I]OVTA binding in saline- and nicotine-treated wild type (WT) and G72 transgenic (G72Tg) mice.*** Brain sections from WT and G72Tg mice treated with chronic saline (SAL) or nicotine (NIC; 24 mg/kg/day) via subcutaneous minipumps for 14 days were bound with [^{125}I]OVTA to determine OTR binding levels in the **(A-C)** anterior olfactory nuclei, **(D)** cingulate, **(E)** piriform and **(F)** endopiriform cortices, **(G)** olfactory tubercle, **(H)** nucleus accumbens, **(I)** caudate putamen, **(J)** lateral and **(K)** medial septum, **(L)** ventral limb of the diagonal band of Broca, **(M)** hippocampus, **(N)** thalamus, **(O)** hypothalamus, **(P)** basolateral, **(Q)** basomedial and **(R)** central amygdala. Data are presented as mean ± standard error of the mean. * $p<0.05$; ** $p<0.01$ (n = 4-10/group).


A
WT Saline
B
WT Nicotine
C
G72Tg Saline
D
G72Tg Nicotine
fmol/mg
Total
NSB
5.379
4.822
2.544
1.150
0.315
0.073
[125I]-OVTA specific binding


Olfactory nuclei
Cortex
Striatum
Septum
Forebrain
Amygdala
A Anterior olfactory nucleus (lateral)
B Anterior olfactory nucleus (ventral)
C Anterior olfactory nucleus (medial)
D Cingluate cortex
E Piriform cortex
F Endopiriform cortex
G Olfactory tubercle
H Nucleus accumbens
I Caudate putamen
J Lateral septum
K Medial septum
L Ventral limb of the diagonal band of Broca
M Hippocampus
N Thalamus
O Hypothalamus
P Basolateral amygdala
Q Basomedial amygdala
R Central amygdala
WT
G72Tg
[125I]-OVTA specific binding (fmol/mg tissue equivalent)
SAL
NIC
0.08
0.09
*
**