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Similar Serotonin-2A Receptor Binding in Rats With Different Coping Styles or Levels of Aggression

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KEY WORDS agonist; antagonist; binding assays; Cimbi-36; frontal cortex; hippocampus; 5-HT_{2A} receptor; individual response; MDL-100907; stress

ABSTRACT Individual differences in coping style emerge as a function of underlying variability in the activation of a mesocorticolimbic brain circuitry. Particularly serotonin seems to play an important role. For this reason, we assessed serotonin-2A receptor (5-HT_{2A}R) binding in the brain of rats with different coping styles. We compared proactive and reactive males of two rat strains, Wild-type Groningen (WTG) and Roman high- and low avoidance (RHA, RLA). 5-HT_{2A}R binding in (pre)frontal cortex (FC) and hippocampus was investigated using a radiolabeled antagonist ([^{3}H]MDL-100907) and agonist ([^{3}H]Cimbi-36) in binding assays. No differences in 5-HT_{2A}R binding were observed in male animals with different coping styles. [^{3}H]MDL-100907 displayed a higher specific-to-nonspecific binding ratio than [^{3}H]Cimbi-36. Our findings suggest that in these particular rat strains, 5-HT_{2A}R binding is not an important molecular marker for coping style. Because neither an antagonist nor an agonist tracer showed any binding differences, it is unlikely that the affinity state of the 5-HT_{2A}R is co-varying with levels of aggression or active avoidance in WTG, RHA and RLA. **Synapse 69:226–232, 2015.** © 2015 Wiley Periodicals, Inc.

INTRODUCTION

Many studies have shown that individuals of the same species differ in the way they cope behaviorally and physiologically with stress. Such differences occur along an axis polarized at the two extremes by proactive or active, and reactive or passive, responses (Koolhaas et al., 2010). A proactive coping style is generally characterized by a high level of active avoidance, aggression, impulsivity and other bold or extravert actions, indicating active attempts to counteract a stressful stimulus. Reactive coping, on the other hand, involves low aggressiveness and impulsivity, immobility and a general tendency to passively accept or introvertedly shy away from a similar stimulus (Coppens et al., 2010). These different behavioral coping styles are associated with distinct autonomic nervous and neuroendocrine (re)activity patterns (Koolhaas et al., 2010). It is generally accepted that individual differences in behavioral and physiological coping style emerge as a consequence of an underlying variability in the activation of a basic mesocorticolimbic brain circuit that includes the prefrontal cortex, amygdala, hypothalamus, hippocampus and their common output projection nodes. The functioning of this network is tightly controlled by brainstem ascending monoaminergic inputs (Coppens et al., 2010; De Boer and Koolhaas, 2003).

Animals with different coping styles show differences in functional sensitivity of the 5-HT_{1A} receptor,

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expressed as autoreceptor on cell bodies in the raphe nucleus and postsynaptically on neuronal terminals (Coppens et al., 2010; De Boer et al., 2005; Koolhaas et al., 2010; Veenema et al., 2005). These results indicate that the responsiveness of the serotonergic system may be related to coping style, and therefore be a characteristic trait of an individual.

Another serotonergic target is the 5-HT$_{2A}$ receptor (5-HT$_{2A}$R). Various clinical studies have shown differences of 5-HT$_{2A}$R binding in human subpopulations, but literature findings are not consistent. Cerebral 5-HT$_{2A}$R binding has been investigated most frequently in relation to aggressiveness, which could be related to general coping style. Violent aggression in humans was reported to be related to a decreased binding potential (BP$_{ND}$) of the PET tracer [^{18}F]setoperone in prefrontal cortex, especially at young age (Meyer et al., 2008). Using another PET tracer, [^{11}C]MDL-100907, reduced 5-HT$_{2A}$R availability was also observed across cortical regions in males with extreme levels of impulsive aggression without callous unemotional traits as compared to males with low levels of impulsivity (Rylands et al., 2012). In contrast to these findings, two other PET studies reported that 5-HT$_{2A}$ receptor binding in the prefrontal cortex is increased in physically aggressive patients with impulsive-aggressive personality disorder (Rosell et al., 2010), and in patients with borderline personality disorder (Soloff et al., 2007) as compared to healthy controls. In addition, a postmortem study indicated that 5-HT$_{2A}$ receptor gene expression in the prefrontal cortex is positively correlated with life-time aggression in subjects who committed suicide, but not in subjects who died from non-neurological causes (Oquendo et al., 2006). However, a recent study using a large sample of healthy individuals did not find a consistent relationship between 5-HT$_{2A}$R binding in frontal cortex and the personality traits aggression or impulsivity (Da Cunha-Bang et al., 2013). As these human studies involved different subject groups and employed different radiotracers for 5-HT$_{2A}$R imaging, results cannot be directly compared. An interesting finding is that 5-HT$_{2A}$R binding in the human brain is related to neuroticism, especially the subscale vulnerability, indicating that the expression level of this receptor may be a trait characteristic (Frokjaer et al., 2008).

The relationship between 5-HT$_{2A}$R binding and aggression has also been studied in experimental animals (Morrison et al., 2011; Popova et al., 2010). In the rodent brain, 5-HT$_{2A}$ receptor expression is particularly high in the frontal cortex. The regional number of binding sites decreases in the following order: frontal cortex > striatum > rest of the cortex > bulbus olfactorius > amygdala > hippocampus > hypothalamus > pons and medulla > cerebellum (Visser et al., 2013). No change in the functional sensitivity of 5-HT$_{2A}$R was found in Norway rats bred for high defensive fear-induced aggression toward man, compared to rats with normal aggression, and 5-HT$_{2A}$R expression was also similar (Popova et al., 2010). The 5-HT$_{2A}$R expression in the hamster brain did not change after social defeat, either in subordinate or dominant animals, as tested by immunohistochemistry (Morrison et al., 2011). It should be noted, however, that the specificity of antibodies for 5-HT$_{2A}$R is questionable (Weber and Andrade, 2010). Single photon emission-computed tomography (SPECT) studies observed differences of 5-HT$_{2A}$R binding in impulsive, aggressive dogs compared to normal dogs. These dogs showed increased 5-HT$_{2A}$R binding in cortical areas, which could be ameliorated by administration of the antidepressant citalopram (Peremans et al., 2003, 2005). In conclusion, the relationship between aggression, coping style and 5-HT$_{2A}$R expression is far from clear.

Both the Wild-type Groningen (WTG) strain which has been bred from animals originating in the wild and the Roman rat strains have been extensively characterized with respect to differences that relate to proactive and reactive behaviour. Such behavioral differences are very evident in these strains. Individual WTG rats display different levels of aggression when they encounter an opponent and show also either proactive (active) or reactive (passive) interactions with a changing environment (De Boer and Koolhaas, 2003; Koolhaas et al., 1999, 2007). Research on the avoidance of a shock by rodents has led to the breeding of the Roman high- and low avoidance rat strains (RHA vs. RLA). The difference in avoidance behaviour of these two genetically-selected strains could be interpreted as RHA being proactive and RLA being reactive copers (Steimer et al., 1997b; Steimer and Driscoll, 2003, 2005). However, when tested in behavioral paradigms for anxiety, the distribution range was different from that observed when animals were tested for coping style. Thus, RHA and RLA differ in both coping style and anxiety, whereas coping and anxiety are independent vectors (Steimer et al., 1997b). This observation is the basis for the two-tier model, wherein emotional reactivity is a separate dimension from coping style. Coping style in the WTG rat is also not correlated with anxiety, as measured in the elevated plus-maze (De Boer and Koolhaas, 2003). While WTG rats are phenotypically selected based on their level of offensive aggression, RHA and RLA rats are genetically selected based on their level of active shock-avoidance. The last two strains do not differ in offensive aggression, but rather in anxiety (Steimer et al., 1997a; Coppens et al., 2012). An early study found an increased binding of the 5-HT$_{2A}$R antagonist [^{3}H]ketanserin in RHA compared to RLA rats (Kulikov et al., 1995b). These data have recently been confirmed in a study using the more selective radioligand ^{3}H-MDL100,907 (Klein

et al., 2014), indicating that 5-HT_{2A}R expression may be related to coping style.

The current study aims to investigate whether the binding of the 5-HT_{2A} antagonist [^{3}H]MDL 100,907 (Johnson et al., 1996; Kristiansen et al., 2005; López-Giménez et al., 1998) and the 5-$HT_{2A/2C}$ agonist [^{3}H]Cimbi-36 (Ettrup et al., 2011) is different in rats displaying different coping styles. The comparison between an antagonist and an agonist may be interesting, as it is hypothesized that an agonist binds mainly to the high affinity state of the receptor, while an antagonist binds to both the low- and high affinity states (Seneca et al., 2006). We decided to use WTG, RHA and RLA rats as animal models because of the unique features of these strains, WTG rats originating from wild animals and Roman rats being bred for desired behavioural characteristics. Moreover, WTG rats show differences in aggression while the Roman rats do not. This difference concerning the parameter aggression makes any comparison between the strains very interesting. Finally, the behaviour of male rats from these strains has been extensively characterized, and both strains are locally available.

MATERIALS AND METHODS

Animals

Two different rat models were used to assess the relationship between coping style and 5HT_{2A}R binding: adult, male WTG rats ($n = 16$), and RHA or RLA rats ($n = 16$, outbred). All animals were 3-months old and bred in our own facilities. Proactive and reactive WTG rats can be selected by assessment of their level of aggression in the resident-intruder test (high- and low aggression levels, respectively), while RHA and RLA animals are selectively bred for their proactive and reactive coping styles, or high- and low shock-avoidance.

All animals were kept under a 12:12 h light:dark cycle with lights on at 19:00 h (as WTG rats were tested for aggression in their dark phase, when they were most active). Food and water were available ad libitum. The animal experiments were performed by licensed investigators in compliance with the Law on Animal Experiments of The Netherlands. The protocol was approved by The Institutional Animal Care and Use Committee of the University of Groningen.

Resident–intruder test

To select aggressive (proactive) and non-aggressive (reactive) rats form the local breeding colony, individual animals were screened for aggression upon reaching adulthood. Twenty-four male WTG rats were housed together with a tubaligated female WTG rat to stimulate territorial aggression. The cage had sliding, plexiglass doors, thus the interaction of animals could be filmed at the time of the resident–intruder test. The female was taken out of the homecage 1 h before a male Wistar intruder was placed in that cage with the male WTG. First, the attack latency of all WTG rats was tested for 3 days. During these tests, the Wistar rat was taken out of the cage after the WTG rat had attacked (full clinch). Second, all WTG rats were characterized for their aggressive behaviour on a fourth day by placing the Wistar in their cage for 10 min. The animals always encountered an unfamiliar opponent. The different behaviours displayed during these 10 min were analyzed by scoring the percentage of time spent on that behaviour. The aggressive behaviours scored were: lateral threat, downkeeping, clinch/attack bite, chase, and upright fight. Other (non-aggressive) behaviours scored were: social exploration, ambulant behaviour, rearing, grooming and inactivity. From the 24 animals tested, the 8 most aggressive and the 8 least aggressive were included in the high- and low aggressivity groups, respectively. The average time spent on aggressive behaviour was $68\% \pm 7\%$ in the high aggressivity group, and $19\% \pm 9\%$ in the low aggressivity group. Brains were collected at least 1 week after the last test. Brains of the Roman rats were also collected.

Brain dissection and homogenization

Blood was removed from the brain by perfusing the animals for 1 min with a solution of heparin in saline ($\sim$10 U ml^{-1}). Frontal cortex (FC) and hippocampus (Hip) were isolated and snap frozen in liquid nitrogen, then stored at -80 °C until further processing.

Brains were homogenized with ten volumes of homogenization buffer (50 mM Tris-HCl, 150 mM NaCl and 20 mM EDTA, pH 7.4)., The homogenate was centrifuged at 33,000*g* for 10 min at 0–2 °C. The supernatant was discarded and the pellet was homogenized in lysis buffer (50 mM Tris-HCl and 5 mM EDTA, pH 7.4). The homogenized pellet was then centrifuged at 1000*g* for 1 min at 0–2 °C. This procedure was repeated and the supernatant of both runs was centrifuged at 33,000 *g* for 10 min at 0–2 °C. The pellet was resuspended in 50 mM Tris-HCl with protease inhibitor (complete tablet, Roche). Protein concentration was determined by a DC Protein Assay Kit (Biorad, Hercules, CA).

Binding assay

[^{3}H]MDL-100907 and [^{3}H]Cimbi-36 were kindly provided by Prof. Christer Halldin (Karolinska Institute, Stockholm, Sweden). Saturation binding to 5-HT_{2A}R was assessed by adding 25 μL of tissue homogenate to six different concentrations (0.06–2 nM) of [^{3}H]MDL-100907 or [^{3}H]Cimbi-36 in 975 μL assay buffer (50 mM Tris-HCl for [^{3}H]MDL-100907 and 50 mM of Tris-HCl with 0.1% BSA and 4 mM $CaCl_2$ for [^{3}H]Cimbi-36). The [^{3}H]MDL100907

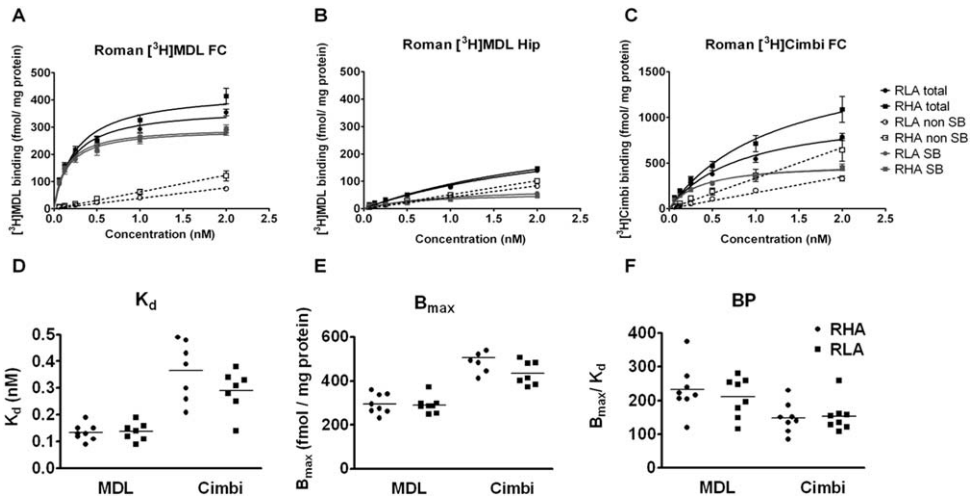

Fig. 1. The 5-HT$_{2A}$ receptor binding in Roman high-avoidance (RHA) and Roman low-avoidance (RLA) rats. Upper row: dose-response plots of A. [^{3}H]MDL-100907 binding in frontal cortex (FC), B. [^{3}H]MDL-100907 binding in hippocampus (Hip), and C. [^{3}H]Cimbi-36 binding in FC. Lower row: Calculated values for D. receptor affinity (K_d), E. maximal number of binding sites (B_{max}) and F. binding potential (B_{max}/K_d = BP in FC. Abbreviations: non SB = nonspecific binding, SB = specific binding.

binding assay was performed as previously described (Kristiansen et al., 2005). The [^{3}H]Cimbi-36 binding protocol was largely based on a previous report using radiolabelled 5-HT$_{2A}$ receptor agonists (López-Giménez et al., 2001), and prior to investigation of [3H]Cimbi-36 binding in RHA/RLA and high/low WTG rats, the assay was validated in wild-type rat tissue (data not shown). Non-specific binding was determined by addition of 10 μM of ketanserin in repeated and adjacent samples. All dilutions were made in duplicate. The samples were incubated on a shaker, in a stove at 37 °C for 90 min. After incubation, the reaction mixture was flushed over a microfiber filter, which was soaked in 1% polyethylenimine solution, using ice cold assay buffer and a harvester. Scintillation fluid (2 mL, Ultima Gold) was added to the filters which were then incubated overnight at 4 °C. The next day, samples were counted in a scintillation counter.

Affinity for the radioligand (K_d) and the maximum number of available binding sites (B_{max}) were calculated from a nonlinear regression analysis of the total- and non-specific binding, using GraphPad Prism 5.0. The binding potential (BP) was calculated as B_{max}/K_d. As [^{3}H]Cimbi-36 has high nonspecific binding, we did not use this ligand for measurement of 5-HT$_{2A}$R numbers in the hippocampus, where the levels of 5-HT$_{2A}$ receptor expression are low. Details concerning the binding assay (e.g. protein concentrations in the assay) can be found in the online version of this article (Additional Supporting Information)

Statistics

Outcome measures of the binding assay were analyzed by a two-tailed student's t test for statistical significance. The level of statistical significance was set at $P < 0.05$.

RESULTS

Binding assay in RHA and RLA

Both [^{3}H]MDL-100907 and [^{3}H]Cimbi-36 demonstrated specific binding to 5-HT$_{2A}$R in FC, as indicated by significant blocking of ligand binding in the presence of saturating concentrations of ketanserin. However, the residual non-specific binding of [^{3}H]Cimbi-36 was higher than that of [^{3}H]MDL-100907 (Fig. 1). The average ratio between total and nonspecific binding at the highest ligand concentration of 2 nM was 15 for [^{3}H]MDL-100907 and 5.5 for [^{3}H]Cimbi-36. ^{3}H]MDL-100907 binding showed a greater variability in Hip than in FC, resulting in greater standard errors in the calculated values for K_d and B_{max}.

RHA and RLA rats did not show any difference in the binding parameters of [^{3}H]MDL-100907 in FC (for K_d $t = 0.21$, Df = 13, $P = 0.84$; for B_{max} $t = 0.23$, Df = 14, $P = 0.82$, see Fig. 1). A similar observation was made in Hip. The B_{max} of [^{3}H]Cimbi-36 tended to be decreased in the FC of RLA rats compared to RHA. However, a two-tailed t test indicated that this difference was not statistically significant ($t = 2.02$, Df = 12, $P = 0.07$). An overview of the outcome measures is presented in Table I.

Binding assay in high- and low aggressive WTG rats

Both [^{3}H]MDL-100907 and [^{3}H]Cimbi-36 showed specific binding to 5-HT$_{2A}$R in the FC of WTG rats, and [^{3}H]Cimbi-36 displayed a higher nonspecific binding also in this strain (Fig. 2). The average ratio between total and nonspecific binding at the highest

TABLE I. 5-HT$_{2A}$ binding assay parameters Roman rats

	Roman high avoidance			Roman low avoidance		
	[^{3}H]MDL		[^{3}H]Cimbi	[^{3}H]MDL		[^{3}H]Cimbi
	FC	Hip	FC	FC	Hip	FC
K_d	0.13 ± 0.03	0.84 ± 0.47	0.37 ± 0.11	0.14 ± 0.03	1.04 ± 0.55	0.29 ± 0.08
B_{max}	296 ± 46	65.0 ± 20	506 ± 75	291 ± 38	79 ± 28	435 ± 54
BP	232 ± 72	9.41 ± 3.91	150 ± 48	210 ± 59	8.39 ± 2.52	160 ± 47

B_{max} = maximum number of available binding sites (fmol mg^{-1} protein), BP = binding potential, FC = frontal cortex, Hip = hippocampus, K_d = affinity (nM). Data presented as mean ± SD.

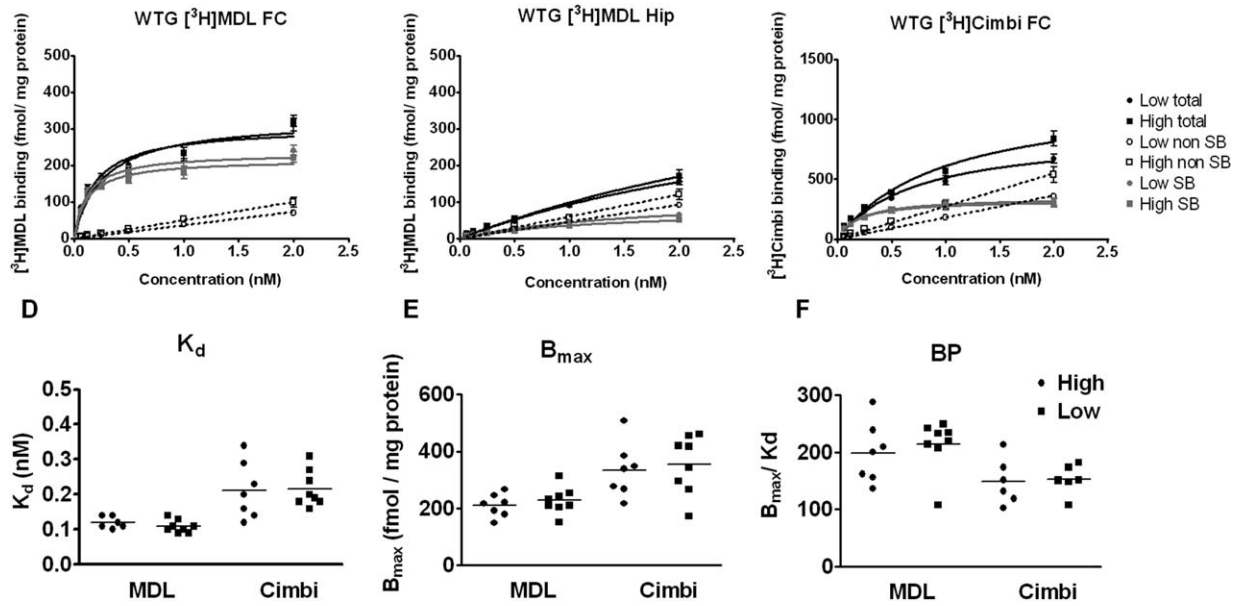

Fig. 2. The 5-HT$_{2A}$ receptor binding in Wild-type Groningen (WTG) rats showing high and low aggression. Upper row: Dose-response plots of A. [^{3}H]MDL-100907 binding in frontal cortex (FC), B. [^{3}H]MDL-100907 binding in hippocampus (Hip), and C. [^{3}H]Cimbi-36 binding in FC. Lower row: Calculated values for D. receptor affinity (K_d, E. maximal number of binding sites (B_{max}) and F. binding potential (B_{max}/K_d = BP) in FC. Abbreviations: non SB = nonspecific binding and SB = specific binding.

ligand concentration of 2 nM was 13 for [^{3}H]MDL-100907 and 5.5 for [^{3}H]Cimbi-36. Low levels of 5-HT$_{2A}$R expression in Hip resulted in higher variation of the K_d and B_{max} values for [^{3}H]MDL-100907 binding in this region.

No significant differences in the parameters of [^{3}H]MDL-100907 binding to the FC of WTG rats with high and low aggression were observed (for K_d $t = 1.19$, Df = 12, $P = 0.26$; for B_{max} $t = 0.77$, Df = 13, $P = 0.46$, see Fig. 2). The same observation (lack of any difference) was made for [^{3}H]MDL-100907 binding in Hip and [^{3}H]Cimbi-36 binding in FC. The outcome measures for the WTG rat strain are presented in Table II.

DISCUSSION

This study investigated whether there are any differences of 5-HT$_{2A}$R binding in animals with different coping styles. Two different animal models: RHA and RLA rats and high- and low aggressive WTG rats, were used as subjects with different coping style. We found similar 5-HT$_{2A}$R binding in the FC or Hip of either strain, both with a radiolabeled antagonist ([^{3}H]MDL-100907) and with an agonist ligand ([^{3}H]Cimbi-36). Thus, our results do not support the hypothesis that differences of 5-HT$_{2A}$R expression are an important factor contributing to differences in coping style in WTG, RHA and RLA rats.

Although [^{3}H]Cimbi-36 binding tended to be increased in RHA compared to RLA, this difference was not statistically significant. If a study in a larger group of animals would confirm the existence of a small difference, this could either mean that the high-affinity state of 5-HT$_{2A}$R (detected by the agonist tracer) is slightly increased in RHA, or that 5-HT$_{2C}$R are somewhat more expressed in RHA than in RLA. [^{3}H]Cimbi-36 may bind to both 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, whereas [^{3}H]MDL-100907 is selective for the 5-HT$_{2A}$R subtype. Lower subtype selectivity of [^{3}H]Cimbi-36 could explain why this ligand showed

TABLE II. 5-HT_{2A} binding assay parameters wild-type Groningen rats

	High aggressivity			Low aggressivity		
	[^{3}H]MDL		[^{3}H]Cimbi	[^{3}H]MDL		[^{3}H]Cimbi
	FC	Hip	FC	FC	Hip	FC
K_d	0.12 ± 0.01	1.53 ± 0.85	0.21 ± 0.08	0.11 ± 0.02	1.78 ± 0.96	0.22 ± 0.05
B_{max}	211 ± 40	93 ± 43	335 ± 95	228 ± 47	122 ± 49	354 ± 103
BP	199 ± 53	6.70 ± 1.74	149 ± 40	214 ± 45	7.52 ± 2.80	152 ± 24

B_{max} = maximum number of available binding sites fmol mg^{-1} protein), BP = binding potential, FC = frontal cortex, Hip = hippocampus, K_d = affinity (nM). Data presented as mean ± SD.

higher B_{max} values than [^{3}H]MDL-100907, since ketanserin pretreatment results in blocking of both 5-HT_{2A} and 5-HT_{2C} receptors (Herndon et al., 1992). However, in a study in the nonhuman primate brain, the binding of [^{11}C]Cimbi-36 in cortical areas was unaltered by selective blockade of 5-HT_{2C} receptors, which indicates that binding of Cimbi-36 to 5-HT_{2C} receptors in frontal cortex is negligible. A small effect of 5-HT_{2C} blockade was noted on [^{11}C]Cimbi-36 binding in hippocampus, most probably due to decreased spill-over from the chorioid plexus, a region very rich in 5-HT_{2C} receptors (Finnema et al., 2014).

Similarity of the binding of [^{3}H]Cimbi-36 in the FC of WTG rats with different levels of aggression suggests that aggression is not related to the affinity state of 5-HT_{2A}R, since agonists are known to bind mainly to the high affinity state of the 5-HT_{2A}R (Fitzgerald et al., 1999). Because of a high nonspecific binding of [^{3}H]Cimbi-36 and a low expression of 5-HT_{2A}R in Hip, it was not possible to acquire reliable values for 5-HT_{2A}R expression with this tracer in this brain area. Because of the low number of hippocampal receptors, such measurements were difficult even with the subtype-selective antagonist [^{3}H]MDL-100907. Data on 5-HT_{2A}R binding show greater variability in Hip than in FC, where the density of 5-HT_{2A}R is much higher (Visser et al., 2013).

In contrast to our negative findings with [^{3}H]MDL-100907 and [^{3}H]Cimbi-36, significant differences of [^{3}H]ketanserin binding in RHA and RLA rats have been reported (Kulikov et al., 1995b). The binding of [^{3}H]ketanserin was also decreased in the striatum of rats and mice genetically predisposed to catalepsy (Kulikov et al., 1995, Popova et al., 1995). Such literature data for [^{3}H]ketanserin cannot be directly compared to our own data for [^{3}H]MDL100907 since [3H]ketanserin may bind to other receptors than 5-HT_{2A}R, viz., histamine H1, alpha-1 and 5-HT_{2C}R. Moreover, these literature data concerned an inbred Roman strain, while in the current study we employed an outbred strain. A recent publication indicates that 5-HT_{2A} receptor expression in the frontal cortex of higher in inbred RHA than inbred RLA (Klein et al., 2014), although the present study shows that it is not different in outbred RLA and outbred RHA. Thus, our negative findings in WTG, outbred RHA and outbred RLA rats can not be generalized to other rodent models of coping style.

Because WTG rats with different levels of aggression showed similar [^{3}H]MDL-100907 binding in FC and Hip, there appears to be no clear relationship between 5-HT_{2A}R binding and aggression in this rat strain. Inconsistent data concerning that relationship have been reported in the literature. Some studies in humans have reported differences of 5-HT_{2A}R binding in subjects with varying levels of aggression (Meyer et al., 2008; Rosell et al., 2010). However, a study in rodents did not find any difference (Popova et al., 2010). In human studies, the subjects were "pathologically" aggressive, whereas the rodents displayed a natural aggressive behavior. Dogs which showed increased 5-HT_{2A}R binding were both aggressive and impulsive (Peremans et al., 2003). Thus, it could be hypothesized that 5-HT_{2A}R levels are changed only in pathologic aggression.

In a follow-up study, 5-HT_{2A}R binding in the rodent brain could be examined before and after a stressful challenge, in order to study the involvement of 5-HT_{2A}R in stress sensitivity. There is evidence in the literature suggesting that 5-HT_{2A}R binding is positively correlated to higher scores for the personality trait neuroticism and especially to vulnerability for stress (risk factors for affective disorders, Frokjaer et al., 2008). Thus, it may be interesting to investigate whether proactive and reactive copers respond differently to stress and if acute stress affects 5-HT_{2A}R binding.

CONCLUSION

We did not observe any difference of baseline 5-HT_{2A}R binding in male rats with different coping styles (RHA, RLA, WTG) and in male rats displaying different levels of aggression (WTG), neither with the radiolabeled antagonist [^{3}H]MDL-100907, nor with the agonist ligand, [^{3}H]Cimbi-36. These data suggest that neither the total number of 5-HT_{2A}R nor the fraction of 5-HT_{2A}R in the high affinity state is covarying with levels of aggression or coping style in males of these rodent strains. Future studies could focus on the effects of a stressful challenge on cerebral 5-HT_{2A}R density in animals with different coping styles.

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