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Effects of repeated risperidone exposure on serotonin receptor subtypes in developing rats

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Abstract

Risperidone is an atypical antipsychotic drug that is widely prescribed to young patients with different psychotic disorders. The long-term effects of this antipsychotic agent on neuronal receptors in developing brain remain unclear and require further investigation. In this study, we examined the effects of long-term treatment of risperidone on two serotonin receptor subtypes in brain regions of juvenile rat. Levels of 5-HT_{1A} and 5-HT_{2A} receptors in forebrain regions of juvenile rats were quantified after 3 weeks of treatment with three different doses of risperidone (0.3, 1.0 and 3.0 mg/kg). Findings were compared to previously reported changes in 5-HT receptors after risperidone treatment (3.0 mg/kg) in adult rat brain. The three doses of risperidone selectively and dose-dependently increased levels of 5-HT_{1A} receptors in medial prefrontal and dorsolateral frontal cortices of juvenile animals. The higher doses (1.0 and 3.0 mg/kg) of risperidone also increased 5-HT_{1A} receptor binding in hippocampal CA_1 region of juvenile but not adult rats. In contrast, the three doses of risperidone significantly reduced 5-HT_{2A} labeling in medial prefrontal and dorsolateral frontal cortices in juvenile as well as in adult animals in an equipotent fashion. 5-HT_{1A} and 5-HT_{2A} receptors in other forebrain regions were not altered by repeated risperidone treatment. These findings indicate that there are differential effects of risperidone on 5-HT_{1A} and 5-HT_{2A} receptors in juvenile animals, and that the 5-HT system in developing animals is more sensitive than adults to the long-term effects of risperidone.

Keywords

Autoradiography; Caudate-putamen; Childhood-onset schizophrenia; Frontal cortex; Risperidone; Serotonin receptors

1. Introduction

Childhood-onset schizophrenia is an uncommon, but severe form of psychotic illness that shares many features of the adult-onset disorder (Gordon et al., 1994; Nicolson and Rapoport,

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Conflict of interest

None of the authors has any financial or non-financial conflict of interest with the findings of this study.

1999). It occurs about 50-times less frequently than adult-onset schizophrenia (Beitchman 1985) when diagnosed by the same criteria applied to adults (Green et al., 1992; Gordon et al., 1994; Spencer and Campbell 1994). Children with schizophrenia can show severe impairments in social and motor development as well as significant premorbid language-delays than those with onset in adolescence (Russell et al., 1989; Alaghband-Rad et al., 1995). Genetic studies confirmed elevated risk of schizophrenia-spectrum disorders in relatives of index cases of childhood-onset schizophrenia compared to families of normal controls or adult-onset schizophrenia patients (Jacobsen and Rapoport, 1998; Nicolson and Rapoport, 1999). Cytogenetic abnormalities are increased in childhood-onset schizophrenia more than in adult-onset schizophrenia, consistent with a neurodevelopmental risk model of the early-onset disorder (Nicolson et al., 1999).

Risperidone (RSP) is widely used for treatment of early onset schizophrenia and other psychotic disorders despite the limited investigation of its efficacy and safety in children and adolescents (Findling and McNamara, 2004). Among the few studies that examined the clinical effects of RSP in pediatric patients, one study found that RSP improved both positive and negative psychotic symptoms in adolescents with schizophrenia (Armenteros et al., 1997), while another study reported that RSP improved cognitive functions in adolescents diagnosed with primary psychotic disorders (Grcevich et al., 1996). RSP was also effective in improving hyperactivity, unstable mood, aggression and self-injurious behaviors in children with pervasive developmental disorders (Perry et al., 1997; Barnard et al., 2002; McCracken et al., 2002; Erickson et al., 2005), and in reducing the severity of motor and vocal tics in patients with Tourette's syndrome (Bruggeman et al., 2001).

Clinical studies have also indicated that pediatric patients are at a higher risk for adverse neurological, hormonal and metabolic effects of RSP than in adult patients. Therefore, defining the optimal therapeutic dose of RSP in young patients is essential to achieve maximal therapeutic efficacy with minimal adverse side effects. In addition, the bioavailability, absorption and metabolism of RSP in children have not been reported, and studies describing the pharmacokinetics of RSP in children and adolescents compared with adults are rare (Grothe et al., 2000; Frazier et al., 2003; McConville and Sorter, 2004). Well-designed clinical trials of RSP and other newer atypical antipsychotic agents are needed to determine their optimal doses, therapeutic effects, and long-term safety in pediatric or adolescent patients diagnosed with idiopathic neuropsychiatric disorders.

RSP targets multi receptor systems with different affinities (Schotte et al., 1996). Similar to other atypical antipsychotic agents, RSP displays a greater affinity for serotonin 5-HT_{2A} than dopamine (DA) D_2 receptors. It also has substantial affinity for DA D_3 and D_4 receptors, adrenergic α_1 and α_2 receptors and histamine H_1 receptors (Schotte et al., 1996). A substantial number of studies have reported on the pharmacological, neurochemical and behavioral properties of RSP (reviewed in Arnt and Skarsfeldt, 1998; Waddington and Casey, 2000). In addition, we quantified the long-term effects of RSP and other antipsychotic drugs on 5-HT receptor subtypes in adult rat brain and reported differential effects on cortical 5-HT_{1A} vs. 5-HT_{2A} receptors (Tarazi et al., 2002). However, the repeated effects of RSP on 5-HT receptors in developing rat brain require investigation to further elucidate the developmental effects of RSP and its influence on brain maturation. Accordingly, we quantified the binding levels of two major 5-HT receptor subtypes (5-HT_{1A} and 5-HT_{2A}) that are known to mediate, at least in part, the actions of modern antipsychotic agents (Baldessarini and Tarazi, 2005), in different forebrain regions after long-term administration of multiple doses of RSP. We also compared the findings to previously reported effects of RSP-induced changes in same 5-HT receptors in adult rat brain (Tarazi et al., 2002).

2. Experimental procedures

2.1. Materials and animal subjects

[±]-2-(N,N-di[2,3-^{3}H]*n*-propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene ([^{3}H]8-OH-DPAT; 154 Ci/mmol) was from Amersham (Arlington Heights, IL); and [ethylene-^{3}H] ketanserin hydrochloride ([^{3}H]ketanserin; 63.8 Ci/mmol) was from New England Nuclear-Perkin-Elmer Corp. (Boston, MA). Kodak biomax MR films and D-19 photographic developer and fixative were from Eastman-Kodak (Rochester, NY).

Janssen Pharmaceutica (Titusville, NJ) donated RSP. Methysergide maleate, pargyline hydrochloride, prazosin hydrochloride, and serotonin hydrochloride were from Sigma-RBI (Natick, MA). Cation hydrochlorides and *tris*-(hydroxymethyl)-aminomethane (Tris) hydrochloride were from Sigma Chemicals (St. Louis, MO).

Subjects were male Sprague-Dawley rats (Charles River Labs., Wilmington, MA) initially weighing 70–80 g at 22 d of age, weaned at 21 d, and maintained under artificial daylight (on, 07:00–19:00 h), in a temp.- and humidity-controlled environment with free access to standard rat chow and tapwater in a USDA-inspected, veterinarian-supervised, small-animal research facility of the Mailman Research Center of McLean Hospital. Animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of McLean Hospital, in compliance with pertinent federal and local regulations.

2.2. In vitro serotonin receptor affinity

RSP was tested for affinity at the 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors in juvenile (PD 30) and adult (PD 90) animals using whole rat brain preparations. Sprague-Dawley rats were sacrificed by decapitation. Brains were quickly removed and dissected on ice. Tissue was homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 150 mM NaCl, washed twice and resuspended in the same buffer. For the 5-HT$_{1A}$ receptor assay, homogenate was incubated with 800 pM [^{3}H] 8-OH-DPAT for 30 min at 30°C; nonspecific binding was defined with 10 μM 5-HT (Taylor et al., 1988). For the 5-HT$_{2A}$ receptor assay, homogenate was incubated with 400 pM [^{3}H] ketanserin for 10–15 min at 30°C; nonspecific binding was defined with 10 μM 5-HT (Leysen et al., 1982). Binding was terminated by immersion in an ice bath. Tissue homogenates were rapidly separated from assay buffer mixtures on glass-fiber filter sheets (ISC BioExpress Co., Kaysville, UT) in a Brandel (Gaithersburg, MD) cell harvester, and washed with excess, ice-cold 150 mM saline. Samples on fiber sheets were punched out as discs and placed in minivials containing 4.5 ml Emulsifier-Safe (Packard Instruments, Meriden, CT), and samples were counted for tritium at 50% efficiency in a Beckman-Coulter liquid scintillation spectrophotometer (Fullerton CA). Assay included more than 10 different concentrations of RSP, in triplicate. IC$_{50}$ ± SE was obtained with the ALLFIT program to fit percent inhibition of specific binding vs. drug concentration, and converted to Ki from the Cheng-Prusoff relationship, Ki = IC$_{50}$/(1 + F/Kd), all as described previously (Kula et al., 1994).

2.3. Drug treatment and tissue preparation

Four groups of rats (N=6/group), at postnatal day 22 [PD 22]), received single, morning (10:00 h) intraperitoneal (i.p.) injections at 2 ml/kg body wt daily for 21 d. The four groups were given RSP in doses of 0.3, 1.0 or 3.0 mg/kg/d, or physiological saline (0.9% w/v) as a control. RSP doses were guided by molecular and in vivo occupancy studies in adult animals (Kusumi et al., 2000; Tarazi et al., 2002; Kapur et al., 2003). No significant changes in body weight were observed after repeated treatment of juvenile animals with three doses of RSP compared to saline-treated animals. After 3 weeks of treatment, juvenile rats were sacrificed 24 hrs after the last injection of RSP or saline (PD 43) by decapitation; brains were removed, quick-frozen in isopentane on dry ice, and stored at −80°C.

Frozen sections (10 μm) were prepared in a cryostat (Microm HM-505E) at −20°C, mounted on gelatin-coated, glass microscope slides, and stored at −80°C until use. Coronal brain sections were taken through medial prefrontal (MPC) and dorsolateral-frontal (DFC) cerebral cortex, nucleus accumbens (NAc), hippocampus (HIP), and medial and lateral caudate-putamen (CPu) [Fig. 1]. These cortical, limbic and extrapyramidal forebrain regions of interest are implicated in cognitive, emotional, and motor behaviors typically disturbed in young and adult patients with psychotic disorders (Baldessarini and Tarazi, 2005).

2.4. Receptor autoradiography

Brain sections from all drug- and saline-treated rats (total 864 sections) were evaluated at the same time in each receptor assay to minimize experimental variability. Sections were first preincubated for 1 h at room temperature (RT) in assay buffer to minimize the effects of endogenous 5-HT and potential interference with residual RSP. Preincubation step is effective in minimizing the effects of endogenous neurotransmitters and potential interference of residual drugs (Florijn et al., 1997).

2.4.1. 5-HT$_{1A}$ receptors—Sections were preincubated for 60 min at RT in 50 mM Tris-HCl buffer (pH 7.6) containing ascorbic acid (0.1%, w/v), $CaCl_2$ (4 mM), and the monoamine oxidase (MAO) inhibitor pargyline-HCl (10 μM). Sections were then incubated for another 60 min at RT in buffer containing 2.0 nM [^{3}H]8-OH-DPAT. Nonspecific binding was defined by 5-HT (1 μM). After incubation, slides were washed (2 × 5 min) in ice-cold buffer and dried under a stream of air (Simpson et al., 1996; Tarazi et al., 2002). The inclusion of Ca^{2+} and the exclusion Na^+ and GTP selectively enhances the binding of [^{3}H] 8-OH-DPAT to high-affinity agonist binding state of 5HT$_{1A}$ receptors (Gilman, 1987).

2.4.2. 5-HT$_{2A}$ receptors—5-HT$_{2A}$ receptors were assayed by our previously reported method (Florijn et al., 1997; Tarazi et al., 2002). [^{3}H]ketanserin was used to label the total 5HT$_{2A}$ receptor population (Teitler et al., 1990). Brain sections were preincubated for 60 min at RT in 50 mM Tris-HCl buffer (pH 7.7), and then incubated for another 60 min at RT in fresh buffer containing 3.0 nM [^{3}H]ketanserin and 1 μM prazosin (to block α_1-adrenoceptors). Nonspecific binding was defined by methysergide (1 μM). After incubation, slides were washed (2 × 30 min) in ice-cold buffer, dipped in ice-cold water, and dried under a stream of air.

2.5. Autoradiography and image analysis

Radiolabeled sections on microscope slides, along with calibrated [^{3}H]standards (Amersham), were exposed to Hyperfilm for 3–4 weeks at 4°C in light-tight cassettes. Films were developed in Kodak developer and fixative. Brain regions of interest were outlined and their optical density (OD) was quantified with a computerized densitometric image analyzer (MCID-M4, Imaging Research; St. Catharines, Ontario). OD was converted to nCi/mg of tissue with calibrated [^{3}H]standards and, after subtracting nonspecific from total binding, specific binding was expressed as fmol/mg tissue (Tarazi et al., 2002).

2.6. Data analysis

Serotonin receptor binding data were analyzed first for overall effects of drug vs. vehicle for all receptor types and brain regions, using multiple regression modeling methods. Density measures were logarithmically transformed to achieve more Gaussian-like distributions prior to regression modeling. Model goodness-of-fit was checked using partial residual plot methods. Since individual brain specimens provided receptor density data for several brain regions, making for incomplete independence across observations, we used robust standard error estimates to adjust for this clustering effect. The estimation method permits relaxation of the

assumption of independence of all observations and requires only that the observations be independent across specimens (Greene, 2000). Estimates of interaction effects were employed for post-hoc tests of drug effects for specific receptors and brain regions, with adjustment of p-values obtained from the regression analyses estimating these multiple comparisons by the standard method of Sidák (Winer et al., 1991). Data are presented as means ± SEM. Comparisons were considered significant at two-tailed $p<0.05$ based on N=6 rats/treatment group.

3. Results

Experiments with rat brain homogenates indicated that RSP exhibits low affinity for 5-HT_{1A} receptors in juvenile and adult animals (Ki > 600 nM; Table 1). In contrast, RSP has very high affinity for 5-HT_{2A} receptors in both aged groups (Ki < 1.7 nM; Table 1). No significant differences were observed in RSP's affinity for either 5-HT_{1A} or 5-HT_{2A} receptors in developing vs. mature animals.

Three weeks of daily injections of 0.3, 1.0 and 3.0 mg/kg of RSP to juvenile rats (from PD 22 to PD 42) significantly and dose-dependently increased binding of [^{3}H]8-OH-DPAT to 5-HT_{1A} receptors in MPC (by 22%, 49% and 75%, respectively; F [df =3; 20] = 11.8, $p<0.001$) and DFC (by 29%, 51% and 67%; F [df=3; 20] = 15.2, $p<0.001$) of juvenile rats (Table 2). In addition, repeated treatment with 1.0 and 3.0 mg/kg RSP increased levels of $5HT_{1A}$ receptors in hippocampal CA_1 region (by 38% and 57%; F [df=3; 20] = 16.1, $p<0.001$) of juvenile rats (Table 2).

Long-term treatment with the three doses of RSP (0.3, 1.0 and 3.0 mg/kg) significantly decreased binding of [^{3}H]ketanserin to $5HT_{2A}$ receptors in an equipotent fashion in the MPC (by 41%, 38% and 36%, F [df=3; 20] = 17.4, $p<0.001$) and DFC (by 40%, 42% and 44%, F [df=3; 20] = 22.0, $p<0.001$) of juvenile rats (Table 3). There were no changes in 5-HT_{2A}-selective labeling in other brain region analyzed after long-term administration of three doses of RSP (Table 3).

4. Discussion

4.1. Effects of risperidone treatment on 5-HT_{1A} receptors

Developmental studies reported that the highest densities of 5-HT_{1A} receptors were found in the cerebral cortex and hippocampus of the human fetal brain between the 16th and 22nd weeks of gestation. These levels were even higher than that reported in cortex and hippocampus of adult human brain (Bar-Peled O et al., 1991). Animal studies found that 5-HT_{1A} receptor expression increased steadily in cerebral cortex, hippocampus and septum from birth till reaching adulthood levels around the third postnatal week (Daval et al., 1987). In our study, we found that long-term administration of 0.3, 1.0 and 3.0 mg/kg of RSP for 21 days significantly and dose-dependently increased 5-HT_{1A} receptor binding in MPC (by 22%, 49% and 75%, respectively), and DFC (by 29%, 51% and 67%) of juvenile rats (age 43 days; Table 2). The significant increases in cortical 5-HT_{1A} receptors in juvenile animals after repeated treatment with RSP parallel the observed increases in 5-HT_{1A} receptors in adult animals after long-term treatment with 3.0 mg/kg of RSP (Table 2; Tarazi et al., 2002).

RSP displayed low affinity for cortical 5-HT_{1A} receptors in both juvenile and adult animals (Ki = 750 nM and 620 nM, respectively, Table 1). This is in agreement with the reported low 5-HT_{1A} receptor-potency of RSP (Ki = 490 nM; Bymaster et al., 1996). The relatively low 5-HT_{1A} receptor-affinity of RSP in juvenile and adult animals suggests that RSP-induced increases in [^{3}H]8-OH-DPAT binding to $5HT_{1A}$ receptors in MPC and DFC reflect indirect mechanisms resulting from more potent effects of RSP on 5-HT_{2A} (Table 2) and D_2 receptors

(Moran-Gates et al., 2007). Microdialysis study in adult animals suggested that RSP-induced increases in cortical DA release resulted from combined blockade of 5-HT_{2A} and D_2 receptors through a 5-HT_{1A}-sensitive stimulatory mechanism and not from direct 5-HT_{1A} receptor activation by RSP (Ichikawa et al., 2001). It is likely that such interactions also occur in juvenile animals and contribute to RSP-induced increases in cortical 5-HT_{1A} receptors

Autoradiographic studies conducted on adult rat brain tissue found that 5-HT_{1A} receptors are highly expressed in rat raphe nuclei, cerebral cortex, and hippocampus, with much lower levels in CPu and NAc (Pazos and Palacios 1985; Pompeiano et al., 1992). In frontal cortex, substantial levels of 5-HT_{1A} receptors are found on neocortical glutamatergic pyramidal neurons that are implicated in cognitive and executive functions (Francis et al., 1992). 5-HT_{1A} receptors have been suggested as neurotherapeutic targets that mediate the beneficial effects of atypical antipsychotic drugs (Millan, 2000). Our findings further support a role for 5-HT_{1A} receptors in mediating actions of RSP and other antipsychotic drugs not only in adults but also in developing animals. It should be noted that postmortem studies reported significant increases in 5-HT_{1A} receptors in frontal cerebral cortex of adult patients diagnosed with schizophrenia (Hashimoto et al., 1993; Burnet et al., 1996; Simpson et al., 1996; Sumiyoshi et al., 1996). Nonetheless, these findings were not reported in young schizophrenia patients, largely owing to the low prevalence of the early-onset disorder and lack of access to postmortem brain tissue from developing patients.

RSP, at 1.0 and 3.0 mg/kg, also increased the abundance of 5-HT_{1A} receptors in the hippocampal CA_1 region of juvenile animals (Table 2). This effect was unique to developing animals as a similar dose of RSP (3.0 mg/kg) failed to alter levels of 5-HT_{1A} receptors in hippocampus of adult animals (Tarazi et al., 2002). This unique age-related receptor and neurochemical differences in RSP's actions suggests that RSP can selectively modulate signal transduction cascades associated with hippocampal 5-HT_{1A} receptors in juvenile and not adult animals.

4.2. Effects of risperidone treatment on 5-HT_{2A} receptors

RSP displayed potent affinity for cortical 5-HT_{2A} receptors in both juvenile and adult animals (Ki = 1.5 nM and 1.2 nM, respectively, Table 1). This is also in agreement with the reported high 5-HT_{2A} receptor-potency of RSP (Ki = 0.6 nM; Bymaster et al., 1996). Three weeks of daily administration of 0.3, 1.0 and 3.0 mg/kg RSP significantly and equipotently decreased binding of [^{3}H]ketanserin to 5HT_{2A} receptors in MPC (by 41%, 38% and 36%, respectively) and DFC (by 40%, 42% and 44%, respectively), and DFC (by 29%, 51% and 67%) of juvenile rats (age 43 days; Table 2). These findings are in agreement with the observed decreases in 5-HT_{2A} receptors in adult animals after continuous treatment with 3.0 mg/kg of RSP (Table 3;Tarazi et al., 2002). It is worthy to note that a low dose of RSP (0.3 mg/kg) reduced cortical 5-HT_{2A} receptor levels in juvenile animals similar to that detected after treatment with a high dose of RSP (3.0 mg/kg) in adult animals (Table 3,Tarazi et al., 2002). These findings further support our hypothesis that developing animals are more sensitive than adults to the long-term neuronal effects of RSP.

Anatomical studies have located 5-HT_{2A} receptors on local GABAergic neurons as well as on corticostriatal glutamatergic pyramidal neurons in frontal cortex (Francis et al., 1992; Wright et al., 1995). Long-term treatment with dissimilar atypical, and not typical, antipsychotic agents reduced 5-HT_{2A} receptor labeling in rat frontal cerebral cortex (O'Dell et al., 1990; Florijn et al., 1997; Kuoppamaki et al., 1995; Kusumi et al., 2000; Tarazi et al., 2002). We have also found that repeated treatment with atypicals clozapine and olanzapine, and not typical agent fluphenazine, decreased 5-HT_{2A} receptor binding in frontal cortex of juvenile animals [PD 43; Gardner et al., in preparation]. The high 5-HT_{2A} receptor-affinity of RSP suggests that the observed decrease in 5-HT_{2A} binding may reflect adaptations to direct 5-HT_{2A} receptor

blockade in both developing and mature animals. Alternatively, and based on the close functional interactions between 5-HT_{2A} and glutamate NMDA receptors (Higgins et al., 2003), it is possible that RSP-induced downregulation of NMDA receptors (Choi et al., 2007) may alter 5-HT neurotransmission and lead to the observed reductions in cortical 5-HT_{2A} receptors. Downregulation of 5-HT_{2A} receptor after long-term treatment with antipsychotic drugs and other 5-HT_{2A} antagonists is a paradoxical effect that contrasts the expected upregulation of neurotransmitter receptors after chronic blockade. Antagonist-induced internalization of 5-HT_{2A} receptors is a possible explanation for this unique molecular phenomenon (Gray and Roth, 2001).

These findings suggest that 5-HT_{2A} receptors constitute one of the common targets that mediate the actions of RSP in juvenile and adult animals. 5-HT_{2A} receptor downregulation may help to compensate for the loss of dopaminergic function in the striatum due to RSP-induced D_2 receptor upregulation and accordingly may lower the incidence of extrapyramidal adverse events and tardive dyskinesia associated with RSP and perhaps other atypical antipsychotics compared with typical antipsychotics in both young and adult patients (Tarsy et al., 2002; Baldessarini and Tarazi 2005). Selective changes in 5-HT_{2A} receptors in cerebral cortex and not in other brain regions examined (Table 3) reflects the differential regional responses of 5-HT_{2A} receptors in juvenile animals to repeated RSP treatment.

4.3. Conclusions

Long-term administration of multiple doses of RSP produced dose-dependent increases in 5-HT_{1A} receptors in MPC and DFC of juvenile animals. Such increases were also observed in adult animals (Table 2). In contrast, repeated treatment with 1.0 and 3.0 mg/kg RSP increased 5-HT_{1A} receptors in hippocampal CA_1 region of juvenile and not adult animals (Table 2). Differences in concentrations of 5-HT_{1A} receptors or magnitude of associated signal transduction cascades evelopmental may have contributed to age-related differences in hippocampal 5-HT_{1A} receptor responses to long-term RSP treatment. Downregulation of cortical 5-HT_{2A} receptors after repeated administration of RSP and other atypical antipsychotic agents suggest that these receptors are common targets that mediate, at least in part, the actions on RSP in developing and mature animals. However, the observed reduction in cortical 5-HT_{2A} receptors in juvenile animals after treatment with a low dose of RSP (0.3 mg/kg) was similar to that observed after treatment with a high dose of RSP (3.0 mg/kg) in adult animals (Table 3). Together, these findings suggest that the 5-HT system in developing animals is more sensitive than adults to the long-term effects of RSP and perhaps other atypical antipsychotic agents.

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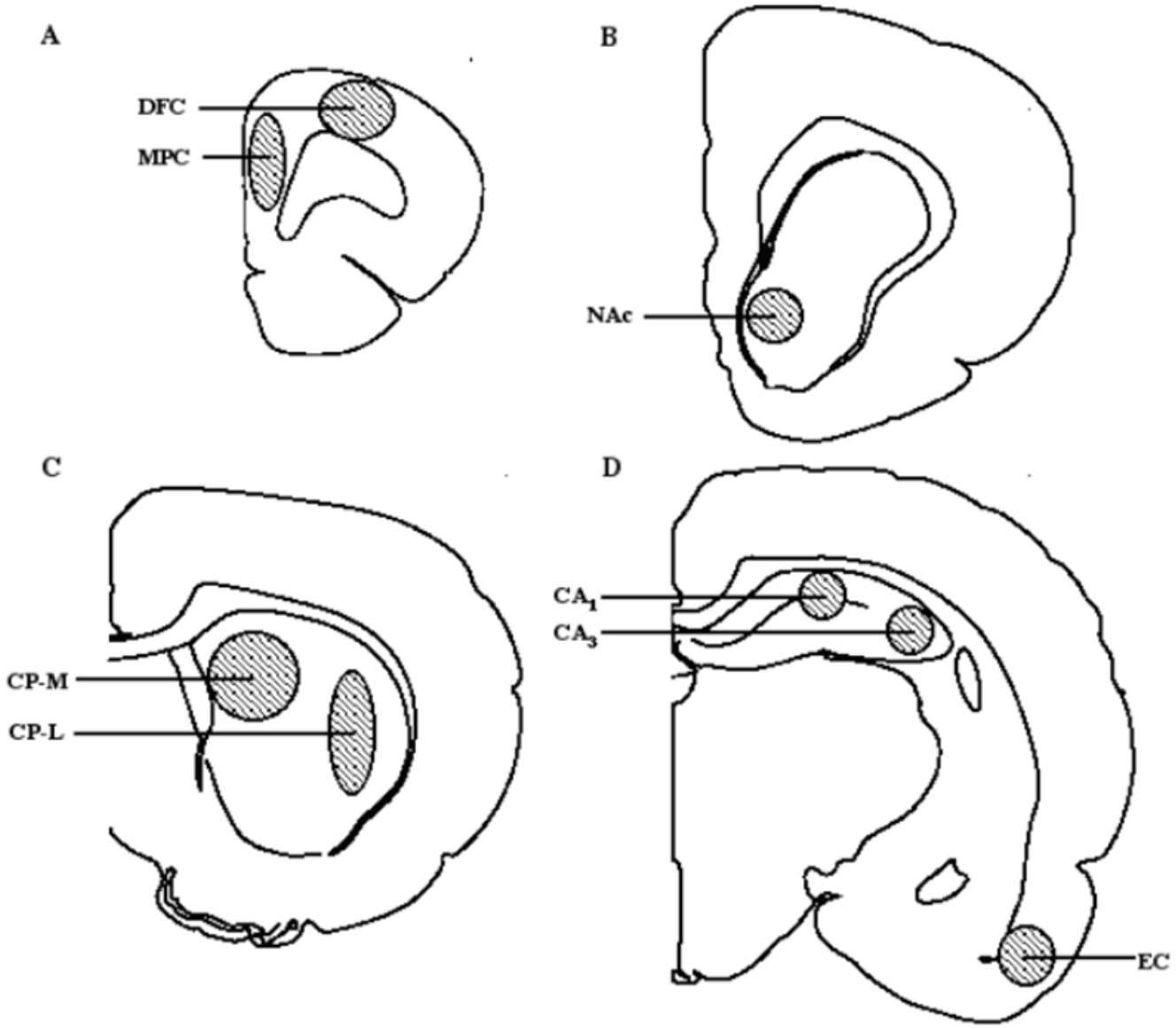

Fig. 1.

Sites of autoradiographic analyses of rat brain regions sampled in 10 μm coronal sections from: A (A 3.2–4.2), B (A 1.7–2.2), C (A 0.7–1.2), and D (A 0.2–0.7 mm anterior to bregma, according to Paxinos & Watson (1982). Abbreviations: NAc, nucleus accumbens septi; CPu, caudate-putamen (L, lateral and M, medial); DFC, dorsolateral-frontal cerebral cortex; EC, entorhinal cortex; CA_1 and CA_3, hippocampal regions; MPC, medial prefrontal cortex.

Table 1

Affinity (K_i, nM ± S.E.) of risperidone at 5-HT_{1A} and 5-HT_{2A} receptors in juvenile vs. adult whole rat brain preparations

Rat brain tissue	5-HT_{1A}	5-HT_{2A}
Juvenile animals (PD 30)	750 ± 44	1.5 ± 0.4
Adult animals (PD 90)	620 ± 58	1.2 ± 0.2

Table 2

5-HT_{1A} receptor binding in juvenile rats (PD 42) after three weeks of daily administration of risperidone

	Adult Rats		*Juvenile Rats*	
Brain region	**Controls (3.0 mg/kg)**	**RSP (0.3 mg/kg)**	**RSP (1.0 mg/kg)**	**RSP (3.0 mg/kg)**
Cerebral cortex				
Medial-prefrontal	34.5 ± 1.0 (100) (**152**)*	42.0 ± 2.3 (**122**)*	51.3 ± 3.3 (**149**)*	60.2 ± 2.0 (**175**)*
Dorsolateral	21.5 ± 1.5 (100) (**173**)*	27.8 ± 1.5 (**129**)*	32.4 ± 2.1 (**151**)*	36.0 ± 2.3 (**167**)*
Nucleus accumbens	4.8 ± 0.3 (100) (119)	4.7 ± 0.3 (98)	5.0 ± 0.5 (104)	5.1 ± 0.4 (106)
Caudate-putamen				
Medial	4.9 ± 0.2 (100) (101)	5.0 ± 0.2 (102)	5.1 ± 0.3 (105)	5.2 ± 0.2 (106)
Lateral	5.4 ± 0.3 (100) (96)	4.9 ± 0.1 (91)	5.3 ± 0.3 (98)	4.8 ± 0.2 (89)
Hippocampus				
CA_1- Region	67.7 ± 5.7 (100) (98)	74.3 ± 6.7 (110)	93.2 ± 7.8 (**138**)*	106.1 ± 9.0 (**157**)*
CA_3- Region	32.4 ± 1.2 (100) (105)	32.0 ± 1.5 (99)	36.1 ± 2.0 (111)	35.2 ± 1.3 (109)
Entorhinal cortex	35.7 ± 2.5 (100) (99)	34.2 ± 3.0 (96)	34.2 ± 3.6 (96)	35.1 ± 2.6 (98)

Data are mean ± SEM values for binding (fmol/mg tissue, [% of control]), determined by quantitative autoradiography following daily i.p. injection of vehicle or risperidone (RSP) for 3 weeks, with significant differences from controls indicated in bold ([*] $p<0.05$, N=6 rats/group). Data (% of control) for RSP (3 mg/kg/d) in adult animals were reported previously (Tarazi et al., 2002) and are shown for comparison.

Table 3

5-HT_{2A} receptor binding in juvenile rats (PD 42) after three weeks of daily administration of risperidone

	ADULT RATS	*JUVENILE RATS*		
Brain region	**Controls (3.0 mg/kg)**	**RSP (0.3 mg/kg)**	**RSP (1.0 mg/kg)**	**RSP (3.0 mg/kg)**
Cerebral cortex				
Medial-prefrontal	80.9 ± 5.3 (100) (**56**)*	48.1 ± 2.5 (**59**)*	50.0 ± 2.7 (**62**)*	51.8 ± 2.4 (**64**)*
Dorsolateral	74.8 ± 4.7 (100) (**69**)*	45.0 ± 2.4 (**60**)*	43.4 ± 3.8 (**58**)*	42.2 ± 2.3 (**56**)*
Nucleus accumbens	20.0 ± 3.1 (100) (98)	17.3 ± 1.4 (87)	17.9 ± 1.9 (90)	18.8 ± 2.9 (94)
Caudate-putamen				
Medial	20.9 ± 3.5 (100) (87)	21.5 ± 3.2 (103)	22.4 ± 2.1 (107)	22.2 ± 3.3 (106)
Lateral	23.3 ± 10.6 (100) (92)	24.6 ± 1.7 (106)	22.2 ± 1.4 (95)	22.8 ± 1.0 (98)
Hippocampus				
CA_1- Region	44.4 ± 3.1 (100) (96)	45.4 ± 3.0 (102)	46.2 ± 3.1 (104)	45.7 ± 2.8 (103)
CA_3- Region	38.2 ± 1.7 (100) (96)	36.0 ± 2.3 (94)	36.2 ± 3.7 (95)	34.7 ± 3.9 (91)
Entorhinal cortex	34.0 ± 3.9 (100) (87)	33.9 ± 2.0 (100)	31.4 ± 1.6 (92)	32.9 ± 1.6 (97)

Data are mean ± SEM values for binding (fmol/mg tissue, [% of control]), determined by quantitative autoradiography following daily i.p. injection of vehicle or risperidone (RSP) for 3 weeks, with significant differences from controls indicated in bold ([*] $p<0.05$, N=6 rats/group). Data (% of control) for RSP (3 mg/kg/d) in adult animals were reported previously (Tarazi et al., 2002) and are shown for comparison.