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Dopamine and Serotonin Receptor Binding and Antipsychotic Efficacy

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The relationship between clinically effective antipsychotic drug dosage and binding affinity to cloned dopamine (DA) and serotonin receptor subtypes was analyzed in an effort to elucidate the contribution of individual receptor subtypes to medication response. Clinically effective dose and binding affinity to D_2 DA receptor were modestly correlated for typical antipsychotic medications ($r = 0.54$, $p = 0.046$), but surprisingly were not correlated for atypical antipsychotics ($r = 0.41$, $p = 0.31$). For typical antipsychotics, a more robust *inverse* relationship was observed between medication dose and 5-HT_{2C} affinity ($r = -0.68$, $p = 0.021$). The strongest correlation for typical antipsychotics was observed between drug dosage and 5-HT_{2C}/D_2 binding affinity ratio ($r = -0.81$, $p = 0.003$). For atypical antipsychotics, no significant correlations were identified between medication dosage and 5-HT_{2C}, 5-HT_{2A}, 5-HT_{2C}/D_2, or 5-HT_{2A}/D_2 receptor-binding affinities. In contrast, atypical antipsychotic medication dosage was highly correlated with the ratios of D_2 (5-HT_{2A}/5-HT_{1A}) ($r = 0.80$, $p = 0.031$), and D_2 (5-HT_{2C}/5-HT_{1A}) ($r = 0.78$, $p = 0.038$) binding affinities. These observations demonstrate an interaction between D_2 and 5-HT_{2C} receptor effects contributing to positive symptom response for typical antipsychotic medications, suggesting that signaling through 5-HT_{2C} receptors interacts with and improves antipsychotic effects achieved via D_2 receptor blockade. This analysis also demonstrates that, in contrast to typical antipsychotics, therapeutic effects of atypical antipsychotic medications are determined by opposing interactions among three different domains: (1) increasing D_2 DA receptor-binding affinity enhances antipsychotic potency. (2) Increasing 5-HT_{2C} and 5-HT_{2A} receptor-binding affinities also facilitate antipsychotic efficacy. (3) Increasing 5-HT_{1A} receptor-binding affinity, in contrast, reduces antipsychotic efficacy.
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INTRODUCTION

Thirty years after the description of a direct linear correlation between dopamine (DA) D_2 receptor-binding affinities and antipsychotic drug potencies for ameliorating psychosis (Seeman *et al*, 1976; Creese *et al*, 1976), this association remains an important cornerstone of current hypotheses of both the etiology and treatment of psychotic disorders (Emilien *et al*, 1999; Seeman, 2002; Seeman and Tallerico, 1998). However, over the past quarter century significant advances have been made in expanding our understanding of receptor-binding affinities and clinically effective drug dosages for a steadily expanding list of antipsychotic medications. These changes may influence the relationships identified in earlier analyses.

First, Civelli *et al*, in cloning the DA D_2 receptor (Bunzow *et al*, 1988) provided the foundation for identifying a family of three receptor subtypes, termed DA D_2, D_3 (Sokoloff *et al*, 1990), and D_4 receptors (Van Tol *et al*, 1991). Together, these receptor subtypes constituted the receptor-binding affinities measured in earlier studies (Seeman *et al*, 1976; Creese *et al*, 1976). Each of these individual receptor subtypes has, in turn, been cloned, and binding data for antipsychotic drugs to these individual cloned DA receptor subtypes and their splice variants, which was not available in 1976, can now be consulted. Variability in antipsychotic medication binding to individual D_2 family receptor subtypes (ie D_2, D_3, and D_4) could help to elucidate the contribution of individual DA receptor subtypes in psychosis.

Secondly, many second-generation antipsychotic medications, which were not available in the 1970s, can now be similarly studied. Only one atypical antipsychotic medication, clozapine, had been released at the time of the original analyses in 1976. Further adding to the rationale for re-

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evaluating the relationship between DA receptor binding and antipsychotic effect is the degree to which D_2 family (Kapur and Remington, 2001) compared with serotonergic (5-HT) receptor binding (Meltzer, 1999) may contribute to the antipsychotic properties of atypical antipsychotic medications.

Third, commonly prescribed doses of antipsychotic medications have changed dramatically over the past 25 years (Baldessarini *et al*, 1993; Vuckovic *et al*, 1990). Although there are few dose-finding studies adequately powered to clearly identify minimally effective antipsychotic drug dose (Zimbroff *et al*, 1997), currently available data (Geddes *et al*, 2000; Bollini *et al*, 1994), as well as revisions in clinical practice, have led to a marked reduction in the average prescribed doses of most typical D_2 receptor antagonists since 1976.

Fourth, cloning and pharmacological characterization of multiple serotonin receptor subtypes, including the 5-HT_{1A} (Fargin *et al*, 1988), 5-HT_{2A} (Pritchett *et al*, 1988), and 5-HT_{2C} (Julius *et al*, 1988) serotonin receptor, provide an opportunity to examine the relationship between antipsychotic binding affinities to these serotonin receptor subtypes and clinically effective antipsychotic drug dosages, an analysis not possible in 1976.

Finally, several frequently prescribed antipsychotic medications, including loxapine and perphenazine, were omitted from the original Seeman analysis, whereas an antidepressant medication (trazodone) was included (Seeman *et al*, 1976). It is therefore of interest to re-examine the correlation between average antipsychotic drug dosages used in the treatment of psychosis and DA and serotonin receptor subtype binding, including all commonly prescribed antipsychotic medications.

METHODS

In order to minimize variability in assay conditions that may lead to differences in K_i value for a given receptor (Strange, 2001), drug affinity K_i values determined by the NIMH Psychoactive Drug Screening Program (Roth *et al*, 2004) were used in our analysis. K_i values selected for analysis were those listed as NIMH Psychoactive Drug Screening Program assay certified data, determined from assays using the cloned human receptors with drugs of interest as test ligands. For K_i values for which PDSP certified assay data were not listed, the average K_i value from assay data compiled on the PDSP web site (Roth *et al*, 2004) using the cloned human receptor with drug of interest as the test ligand was utilized. K_i values from cloned human receptor for three drug/receptor combinations not listed in the PDSP database were identified from published literature. K_i values used in our analysis, with data source, are listed in Tables 1 and 2. As noted, all of the binding data analyzed in our study has been previously reported by other investigators.

Average daily antipsychotic drug dose was determined from data in randomized, controlled clinical trials wherever possible (Leucht *et al*, 1999), supplemented by the consensus clinical experience of three psychiatrists (NMR, PEK, SMS) who regularly prescribe these medications. The drug dosage ranges determined by consensus clinical experience are similar in each case to recommended dosage ranges for published drug reference guides, including ePocrates Rx (ePocrates, San Carlos, California); the Physicians' Desk Reference (Kaplan *et al*, 1994; Bernstein, 1988). The midpoint of the dose range was used in subsequent calculations. Values for antipsychotic drug dose were established before any data analysis, blind to specific K_i values, and are listed in Tables 1 and 2.

Data Analysis

Antipsychotic doses and binding affinities were log-transformed before analysis. Linear regression was used to estimate the association between these quantities. A binary variable indicating antipsychotic class (typical or atypical) was also entered into the model and allowed to interact with (log) dose, so that a different regression equation could be estimated for each antipsychotic class. A test of whether the interaction term was statistically different from zero was then used to determine if separate regression equations were indicated for each antipsychotic class. A statistically significant interaction would allow rejecting the possibility that the relationship between binding and effective dosage was the same for both antipsychotic classes. It should be noted, however, that the statistical power of these tests to detect cases where class-specific relationships exist may be low, because of the limited number of data points. Consequently, failure to detect a statistical difference between the best-fitting regressions for each antipsychotic class should not be taken as proof that such a difference does not exist.

Data were analyzed using separate univariate analyses for each receptor subtype. It was not feasible to analyze data using multivariate methods because the limited number of drugs for which K_i values were available for all receptor subtypes examined did not allow for a meaningful statistical analysis.

The linear correlation coefficient (r) is reported as a standardized measure of strength of association for the regressions within each class and, in cases where equality of the regressions could not be statistically rejected, for an additional regression based on combining data from both classes.

RESULTS

The correlation between average clinically effective antipsychotic dose and binding affinity to the cloned human D_2 receptor is illustrated in Table 3 and Figure 1. Clinically effective dose and binding affinity to D_2 DA receptor were directly correlated for typical antipsychotic medications ($r = 0.54$, $p = 0.046$), but not for second-generation antipsychotic medications ($r = 0.41$, $p = 0.311$). In testing for a difference between the relationships for first- and second-generation drugs, an equality of regression parameters could not be statistically rejected (interaction $p = 0.72$). Combining typical and atypical medications into a single analysis resulted in a linear correlation with $r = 0.48$, $p = 0.023$.

The relationship between average clinically effective dose and binding affinity to the cloned human D_3 receptor is also shown in Table 3. There are no clear correlations between these variables for typical ($r = 0.33$, $p = 0.292$) or atypical

Table 1 Antipsychotic Medication Dopamine Receptor K_i Values

Drug	Clininically effective dose (mg)	K_i Values (nM)								
		D_1	D_2	D_2 short	D_2 long	D_3	D_4	$D_{4.2}$	$D_{4.4}$	D_5
Amisulpride	400–800		*1.3*			*2.4*	*>1000*			
Aripiprazole	5–30	387	0.95		*0.74*	4.5	*>1000*			1676
Benperidol	12–16		*0.027*				*0.066*			
Chlorpromazine	300–900	112	2.0		*5.4*	5.0	*10.8*	*26.2*	*15.9*	133
Chlorprothixene	50–400		*3.3*				*0.64*			
Clozapine	300–900	189	431	*143.3*	*196*	646	*22.5*	*45.2*	*30*	235
Fluphenazine	2–15	24	0.54			3	*35*			12
Haloperidol	2–15	83	2	*1.21*	*2.34*	12	*3.88*	*6.93*	*15*	147
Loxapine	25–100	54	10		*22.3*	30	*10.9*	*14*	*5.9*	75
Mesoridazine	100–400		*4.3*			*2.6*	*9.1*			
Molindone	20–100		*17.8*			*47.7*	*3433*			
Olanzapine	10–20	58	72	*34.6*	*33.2*	63	*17.1*	*44.2*	*40.5*	90
Perphenazine	8–64		*0.56*			*0.43*	*28.5*			
Pimozide	2–10	*>10000*	*2.51*			*2.84*	*1.8*			
Quetiapine	250–800	712	567	*555*	*702*	483	*2276*	*1233*		1738
Remoxipride	200–400		*243*		*125*	*1109*	*2527*			
Risperidone	2–8	60.6	4.9	*4.73*	*6.0*	12.2	*7.12*	*16.7*	*26.3*	16
Sertindole	12–24		*4.14*	*5.8*	*4.87*	*5.76*	*9.29*	*17.67*		
Thioridazine	200–800	89	10	*8.6*		53	*10.65*			216
Thiothixene	5–30	51	1.4			185	***6.4***	*548*		261
Trifluoperazine	5–30		*1.12*			***0.45***	*38*	*178*		
Ziprasidone	80–160	30	4.0	*4.2*	*4.6*	17	*500.8*	*35.3*		152

Normal font = PDSP certified data.
Italic font = PDSP K_i database mean.
Italic underlined = (Schoemaker *et al*, 1997).
Italic bold = (Seeman and Tallerico, 1998).

antipsychotic medications ($r = 0.42$, $p = 0.287$) analyzed separately. Combining typical and atypical medications into a single analysis yielded a similar degree of correlation ($r = 0.37$, $p = 0.100$).

The relationship between average clinically effective antipsychotic dose and binding affinity to the cloned human D_4 receptor is shown in Table 3. For typical antipsychotic drugs, there was no correlation between these two measures ($r = 0.24$, $p = 0.390$). In contrast, for atypical antipsychotics the two measures were more moderately correlated ($r = 0.59$, $p = 0.123$). Equality of regression parameters could not be statistically rejected, however, in testing for a difference between the relationship for first- and second-generation drugs (interaction $p = 0.30$). Combining typical and atypical medications into a single analysis did not demonstrate a significant correlation between the two variables ($r = 0.37$, $p = 0.088$).

The relationship between average clinically effective antipsychotic dose and binding affinity to the cloned human 5-HT_{1A} receptor is shown in Table 3. There were no detectable direct relationships between clinically effective antipsychotic dose and receptor-binding affinity for typical ($r = 0.14$, $p = 0.684$) or atypical antipsychotic medications ($r = -0.14$, $p = 0.791$).

The relationship between average clinically effective antipsychotic dose and binding affinity to the cloned human 5-HT_{2A} receptor is shown in Table 3 and Figure 2. There is no direct relationship between clinically effective antipsychotic dose and receptor-binding affinity for typical antipsychotic medications ($r < -0.10$, $p = 0.812$). In contrast, for atypical antipsychotic medications binding affinity and clinically effective dose were non-significantly correlated, with a moderate effect size ($r = 0.62$, $p = 0.133$). In testing for a difference between these two relationships, the equality of these regression parameters could not be rejected (interaction $p = 0.14$). Combining typical and atypical medications into a single analysis eliminated the correlation between the two measures ($r = 0.17$, $p = 0.514$).

The relationship between average clinically effective dose and binding affinity to the cloned human 5-HT_{2C} receptor is shown in Table 3 and Figure 3. For typical antipsychotic drugs, binding affinity and clinically effective dose were surprisingly *negatively* correlated ($r = -0.68$, $p = 0.021$, Figure 3a). For atypical antipsychotic medications, the direction of the correlation was *opposite* (Figure 3b), and there was no significant direct relationship between clinically effective antipsychotic dose and binding affinity

Table 2 Antipsychotic Medication Serotonin Receptor K_i Values

Drug	Clininically effective dose (mg)	K_i Values (nM)											
		5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	5-HT_{1E}	5-HT_{1F}	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_3	5-$HT5_A$	5-HT_6	5-HT_7
Aripiprazole	5–30	5.6	833	63	8000		*17.5*	*0.36*	*22.4*	628	1241	574	10
Chlorpromazine	300–900	3115	1489	452	344		*3.32*		*15.55*	977	118	12	21
Chlorprothixene	50–400						*0.43*						
Clozapine	300–900	105	398	2132	966	*130*	*9.15*	*7.38*	*14.9*	241	3857	17	18
Fluphenazine	2–15	145	334	334	540		*21*		*983*	>10000	145	28	8
Haloperidol	2–15	1202	165	7606	>10000	*>5000*	*118.6*	*1204*	*5580*	>10000	2247	3666	378
Loxapine	25–100	2456	388	3468	1399		*4.38*		*13.3*	190	776	33	88
Mesoridazine	100–400								*157*			*380*	
Molindone	20–100	*3797*					*4653*		*10000*			*1008*	*3053*
Olanzapine	10–20	2063	509	1582	2408	*310*	*4.90*	*11.8*	*14.2*	202	1212	6.0	105
Perphenazine	8–64	*421*					*5.6*		*132*			*17*	*23*
Pimozide	2–10	*650*					*48.35*		*2112*			*71*	*0.5*
Quetiapine	250–800	431	1109	>10000	2402	*2240*	*526*		*1843*	>10000	3120	1864	308
Remoxipride	200–400						*6225*						
Risperidone	2–8	427	53.6	29.2	>10000	*1240*	*0.481*	41.6	*33.4*	>10000	205.8	2241	6.6
Sertindole	12–24	*280*	*60*	*96*	*430*	*360*	*0.387*		*0.9*			*5.4*	*28*
Thioridazine	200–800	108	109	579	194		*21.5*		*53*	>10000	364	57	99
Thiothixene	5–30	410	151	659	>10000		*50*		*1356*	1863	361	208	15
Trifluoperazine	5–30	*950*					*74*		*378*			*144*	*291*
Ziprasidone	80–160	76	4	9	1279		*0.73*		*13*	>10000	291	61	6

Normal font = PDSP certified data.
Italic font = PDSP K_i database mean.

Table 3 Correlation Between Clinically Effective Antipsychotic Dose and Receptor Binding Affinity

	D_2			D_3			D_4			5-HT_{1A}			5-HT_{2A}			5-HT_{2C}		
Drug	**n**	**r**	***p*-value**	**n**	***r***	***p*-value**	**n**	***r***	***p*-value**	**n**	***r***	***p*-value**	**n**	***r***	***p*-value**	**n**	***r***	***p*-value**
Typical	**14**	**0.54**	**0.046**	12	0.33	0.292	14	0.24	0.390	10	0.14	0.684	12	−0.08	0.812	**11**	**−0.68**	**0.021**
Atypical	8	0.41	0.311	8	0.42	0.287	8	0.59	0.123	7	−0.14	0.791	7	0.62	0.133	7	0.47	0.285
Combined	**22**	**0.48**	**0.023**	20	0.37	0.100	22	0.37	0.088	17	−0.05	0.856	19	0.17	0.514	18	−0.20	0.402

Bold font indicates *p*-value < 0.05.

($r = 0.47$, $p = 0.285$). In testing for a difference between these two relationships, the regression parameters were significantly different (interaction $p = 0.020$). As would be expected, combining typical and atypical medications into a single analysis eliminated any correlation between clinically effective dose and 5-HT_{2C} receptor binding ($r = -0.20$, $p = 0.40$).

In order to evaluate possible interactions between receptor subtypes playing a role in mechanism of antipsychotic efficacy, we analyzed correlations between log (average dose) and log (ratio of binding affinities) for combinations of individual receptor subtypes, as shown in Table 4. As illustrated in Figure 4a, there is a modest negative correlation between average clinically effective dose and ratio of binding affinities for 5-HT_{2A}/D_2 receptors for typical antipsychotic medication ($r = -0.52$, $p = 0.082$), however, surprisingly for atypical antipsychotics, there was no detectable relationship between dose and 5-HT_{2A}/D_2 binding affinity ratio ($r = -0.08$, $p = 0.869$, Figure 4b).

As shown in Figure 5a, typical antipsychotic medication dose and 5-HT_{2C}/D_2 receptor-binding affinity ratios were strongly and inversely correlated ($r = -0.81$, $p = 0.003$). In contrast, there was no detectable relationship between dose and 5-HT_{2C}/D_2 receptor-binding affinity ratio for second-generation antipsychotic medications ($r = -0.30$, $p = 0.507$, Figure 5c).

A similar analysis of 5-HT_{2A}/D_3 receptor-binding affinity ratios did not identify correlations between these values and clinically effective dosages of typical ($r = -0.40$, $p = 0.216$) or atypical ($r < 0.01$, $p = 0.997$), or pooled first- and second-generation antipsychotic medications, as illustrated in Table 4.

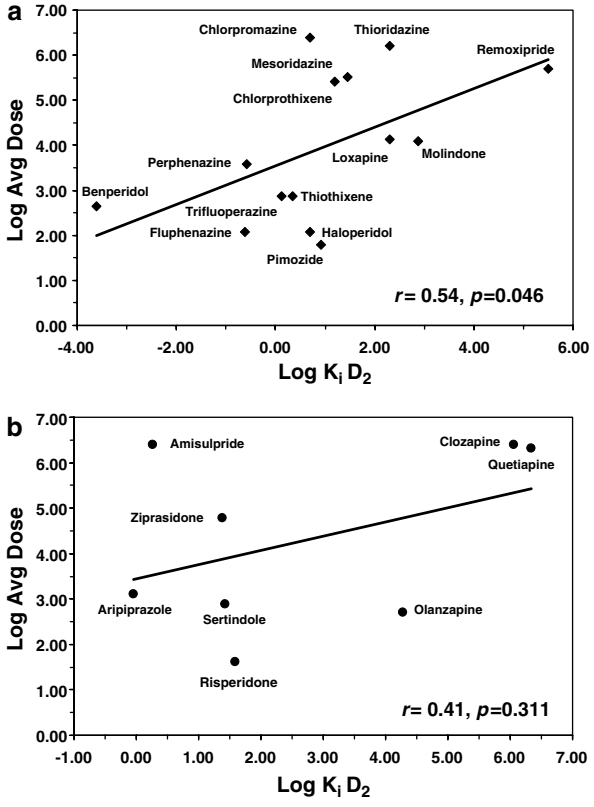

Figure 1 Clinically effective antipsychotic dose *vs* binding affinity to cloned human DA D_2 receptor for (a) typical and (b) atypical antipsychotic medications.

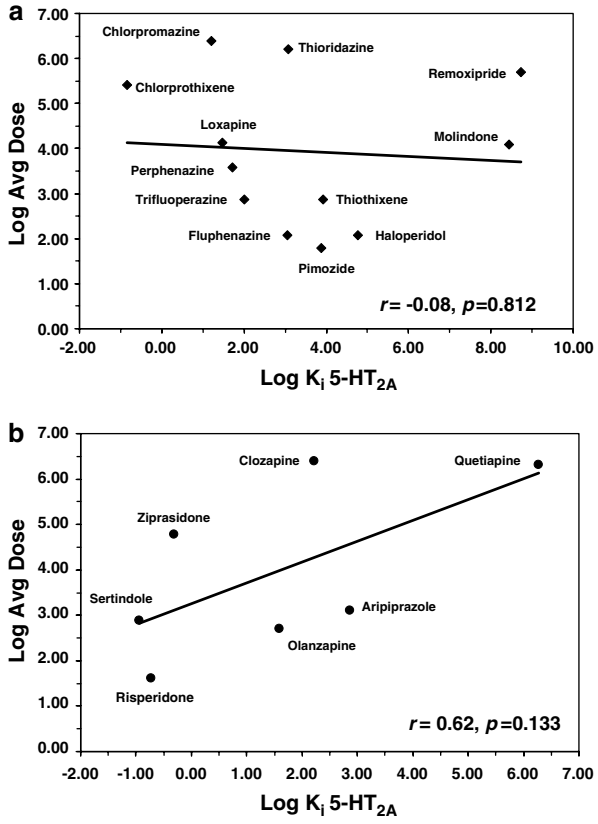

Figure 2 Clinically effective antipsychotic dose *vs* binding affinity to cloned human serotonin 5-HT_{2A} receptor for (a) typical and (b) atypical antipsychotic medications.

In contrast, 5-HT_{2C}/D_3 receptor-binding affinity ratios were correlated with clinically effective dosages of typical antipsychotic medications ($r = -0.67$, $p = 0.023$, Figure 6a). 5-HT_{2C}/D_4 receptor-binding affinity ratios were similarly correlated with clinically effective dosages of typical antipsychotic medications ($r = -0.70$, $p = 0.016$, Figure 7a). A comparable relationship, however, was not observed for atypical antipsychotic medications, as 5-HT_{2C}/D_3 (Figure 6b) and 5-HT_{2C}/D_4 (Figure 7b) receptor-binding affinity ratios were not correlated with clinically effective dosages of atypical antipsychotic medications.

Analysis of DA and serotonin receptor-binding affinities singly and in simple combinations did not identify correlations between receptor subtype binding and clinical efficacy for atypical antipsychotic medications. We therefore further evaluated the relationship between receptor binding and efficacy using a more comprehensive set of binding affinity ratios. Although there is not a universal consensus on this point, it has previously been suggested that the antipsychotic effect of atypical antipsychotic medications results from a balance of inhibition at serotonin 5-HT_{2A}, 5-HT_{2C}, and DA D_2 receptors (Meltzer, 1989; Meltzer, 1995; Leysen *et al*, 1993; Huttunen, 1995), coupled with simultaneous agonist effects at serotonin 5-HT_{1A} receptors (Meltzer, 1999; Millan, 2000; Protais *et al*, 1994). In order to identify therapeutic benefit resulting from the interaction between simultaneous effects at these receptor subtypes, we determined the relationship between clinically effective antipsychotic medication dose and ratios incorporating the binding affinities for each of these receptor systems. As shown in Table 4 and Figure 8 (lower panel), atypical antipsychotic medication dose and D_2 (5-HT_{2A}/5-HT_{1A}) binding affinity ratio are highly correlated ($r = 0.80$, $p = 0.031$). Similarly, D_2 (5-HT_{2C}/5-HT_{1A}) binding affinity ratio and atypical antipsychotic medication dose are also highly correlated ($r = 0.78$, $p = 0.038$, Figure 9, lower panel). In contrast, neither binding affinity ratio is significantly correlated with clinically effective dose for typical antipsychotic medications (Figures 8 and 9, upper panels). Removing the 5-HT_{1A} receptor-binding affinity term from the equation by correlating antipsychotic medication dose with (5-$HT_{2A} \times D_2$) or (5-$HT_{2C} \times D_2$) binding affinity ratio lessens the resulting degree of correlation (Table 4). Similarly, the receptor-binding relationships can be modified, so that 5-HT_{1A} and D_2 receptor binding no longer have functionally opposite roles, and D_2 binding no longer has a functionally similar action as 5-HT_{2A} and 5-HT_{2C} binding, by inverting the serotonin receptor affinity terms (Table 4, lower right two columns). This modification completely eliminates the correlation between binding affinity ratio and drug dosage for atypical antipsychotic medications. Typical antipsychotic drug dosage, in contrast, is significantly correlated with the resulting binding affinity ratio D_2 (5-HT_{1A}/5-HT_{2C}) ($r = 0.75$, $p = 0.013$). Comparing this result to the 5-HT_{2C}/D_2 binding affinity *vs* typical antipsychotic drug dosage

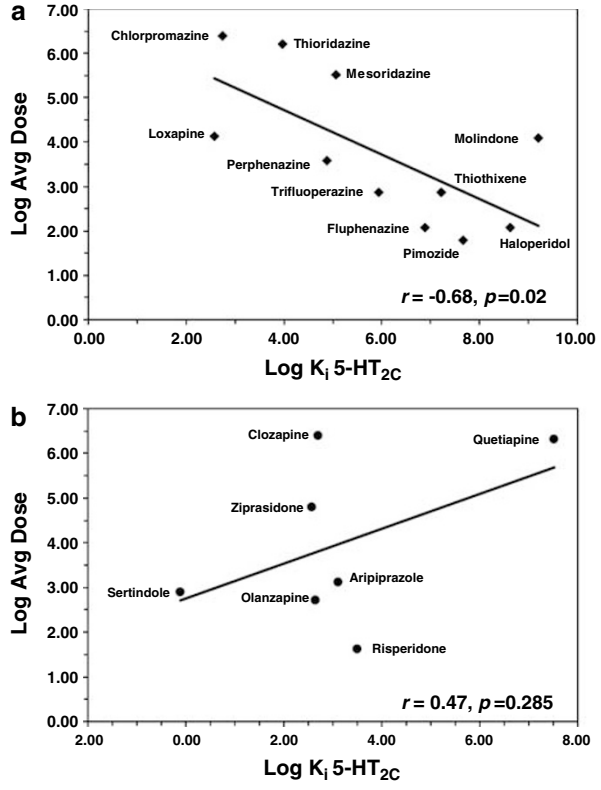

Figure 3 Clinically effective antipsychotic dose *vs* binding affinity to cloned human serotonin 5-HT_{2C} receptor for (a) typical and (b) atypical antipsychotic medications.

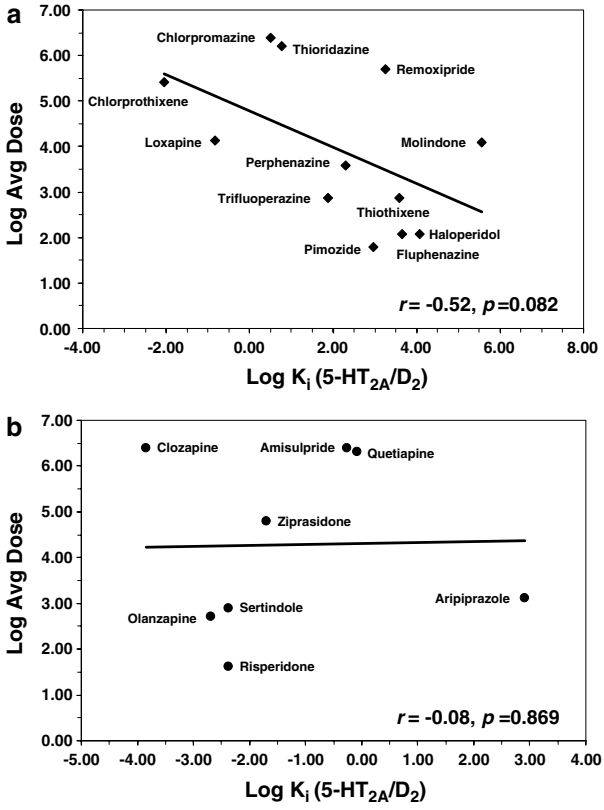

Figure 4 Clinically effective antipsychotic dose *vs* ratio of binding affinity to cloned human serotonin 5-HT_{2A}/DA D_2 receptor for (a) typical and (b) atypical antipsychotic medications.

Table 4 Correlation Between Clinically Effective Antipsychotic Dose and Receptor Binding Affinity Ratios

	$5\text{-}HT_{2A}/D_2$			$5\text{-}HT_{2C}/D_2$			$5\text{-}HT_{2A}/D_3$			$5\text{-}HT_{2C}/D_3$			$5\text{-}HT_{2A}/D_4$			$5\text{-}HT_{2C}/D_4$		
Drug	**n**	***r***	***p*-value**	**n**	***r***	***p*-value**	**n**	***r***	***p*-value**	**n**	***r***	***p*-value**	**n**	***r***	***p*-value**	**n**	***r***	***p*-value**
Typical	12	−0.52	0.082	11	**−0.81**	**0.003**	11	−0.40	0.216	11	**−0.67**	**0.023**	12	−0.39	0.206	11	**−0.70**	**0.016**
Atypical	7	−0.08	0.869	7	−0.30	0.507	7	<0.01	0.997	7	−0.26	0.554	7	0.17	0.702	7	−0.09	0.844
Combined	19	−0.28	0.25	18	−0.47	0.047	18	−0.22	0.378	18	−0.44	0.074	19	−0.14	0.603	18	−0.39	0.112

	$5\text{-}HT_{2A} * D_2$			$5\text{-}HT_{2C} * D_2$			D_2 ($5\text{-}HT_{2A}/5\text{-}HT_{1A}$)			D_2 ($5\text{-}HT_{2C}/5\text{-}HT_{1A}$)			D_2 ($5\text{-}HT_{1A}/5\text{-}HT_{2A}$)			D_2 ($5\text{-}HT_{1A}/5\text{-}HT_{2C}$)		
Drug	**n**	***r***	***p*-value**	**n**	***r***	***p*-value**	**n**	***r***	***p*-value**	**n**	***r***	***p*-value**	**n**	***r***	***p*-value**	**n**	***r***	***p*-value**
Typical	12	0.17	0.594	11	−0.39	0.232	10	−0.04	0.918	10	−0.46	0.180	10	0.54	0.104	10	**0.75**	**0.013**
Atypical	7	0.74	0.059	7	0.68	0.095	7	**0.80**	**0.031**	7	**0.78**	**0.038**	7	0.00	0.974	7	0.13	0.775

Bold font indicates *p*-value < 0.05.

correlation described above (Table 4) suggests that the D_2/5-HT_{2C} term contributes the majority of influence to this relationship.

DISCUSSION

Here, we present data evaluating the relationship between binding affinity to several catecholamine receptor subtypes and drug dosage for antipsychotic efficacy. Our analysis is similar in concept to prior studies demonstrating a linear correlation between antipsychotic drug dose and D_2-family DA receptor-binding affinity (Seeman *et al*, 1976; Creese *et al*, 1976). Our goal was to evaluate additional DA and serotonin receptor subtypes, which had not been identified at the time of the earlier analyses, in order to determine whether affinity to individual receptor subtypes could be correlated with antipsychotic potency of these medications.

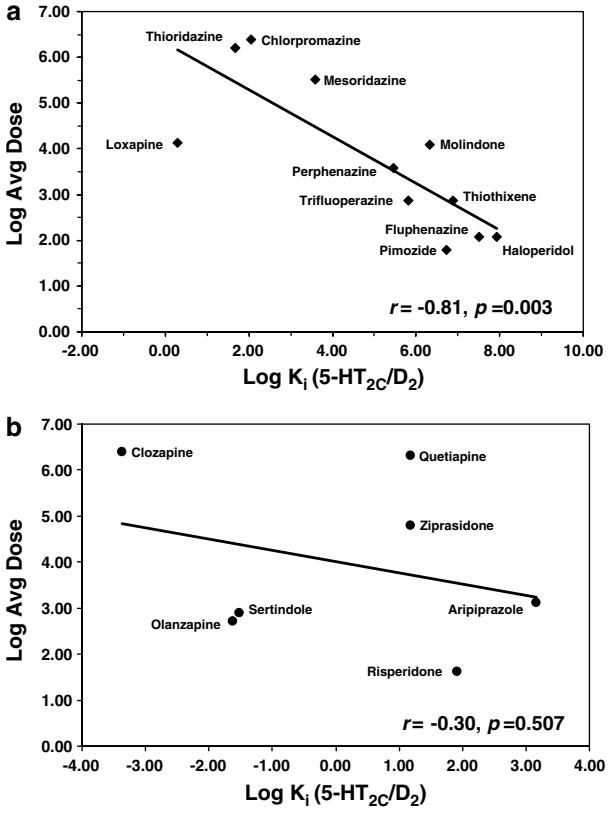

Figure 5 Clinically effective antipsychotic dose *vs* ratio of binding affinity to cloned human serotonin 5-HT_{2C}/DA D_2 receptor for (a) typical and (b) atypical antipsychotic medications.

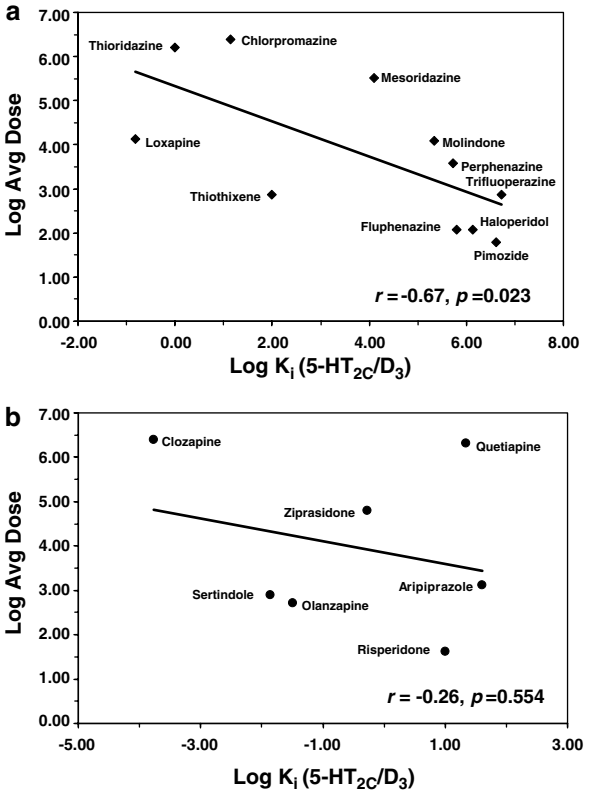

Figure 6 Clinically effective antipsychotic dose *vs* ratio of binding affinity to cloned human serotonin 5-HT_{2C}/DA D_3 receptor for (a) typical and (b) atypical antipsychotic medications.

Although we expected to identify common DA receptor subtypes mediating antipsychotic efficacy for both typical and atypical antipsychotic medications, our analysis instead identified surprising differences in serotonergic mechanisms mediating antipsychotic efficacy for typical *vs* atypical medications. The major findings identified by analysis of this data are discussed below.

Typical Antipsychotic Medications

In agreement with earlier studies, we determined that antipsychotic drug dosage for typical antipsychotic medications is directly correlated with binding to D_2 DA receptors, however, the strength of this correlation was less robust than anticipated. Our data suggest that this may be related in part to interactions between typical antipsychotic medications and serotonin 5-HT_{2C} receptors. The observation that serotonin 5-HT_{2C} receptor affinity is *negatively* correlated with antipsychotic drug dosage for *typical* antipsychotic medications was an unexpected outcome of our data analysis. Additionally, 5-HT_{2C} and D_2 receptor-binding affinities of typical antipsychotic medications interact such that the ratio of serotonin 5-HT_{2C}/D_2 receptor-binding affinity more accurately predicts dosage needed for antipsychotic effect than do 5-HT_{2C} or D_2 binding affinities independently. Thus, increasing serotonin 5-HT_{2C} receptor antagonist affinity lowers antipsychotic potency at any given level of D_2 blockade, suggesting that signaling through 5-HT_{2C} receptors interacts with and improves antipsychotic effects achieved via D_2 receptor blockade. In contrast, the correlation between serotonin 5-HT_{2C} receptor affinity and clinically effective antipsychotic drug dose for *atypical* antipsychotic medications differs from the correlation for typical medications ($p = 0.02$), is in the opposite direction, and the degree of correlation is less pronounced (Figure 3). Although a potential role for 5-HT_{2C} receptor *antagonism* in the therapeutic effect of *atypical* antipsychotic medications has previously been discussed (Meltzer *et al*, 2003; Meltzer, 1995; Wood *et al*, 2006), it has also been suggested that 5-HT_{2C} *agonism* could be therapeutic (Meltzer, 1999; Marquis *et al*, 2007) based on a wide range of preclinical measures demonstrating that serotonin 5-HT_{2C} receptor stimulation inhibits the mesolimbic DA system (Alex *et al*, 2005; Pozzi *et al*, 2002; Di Giovanni *et al*, 1999; De Deurwaerdere and Spampinato, 1999; Millan *et al*, 1998; Di Matteo *et al*, 1999; Di Matteo *et al*, 2002). These findings are consistent with our observation, and suggest a potential mechanism for 5-HT_{2C} receptor blockade to worsen psychotic symptoms. Human data supporting the concept that 5-HT_{2C} blockade lowers the antipsychotic potency of first-generation antipsychotic medications has not been previously elucidated to our knowledge, however.

The neuroanatomical mechanism(s) underlying this finding may be related to the tonic inhibitory control exerted by serotonin operating through 5-HT_{2C} receptors over limbic dopaminergic pathways (De Deurwaerdere and Spampinato, 1999). Serotonergic cell bodies originating in

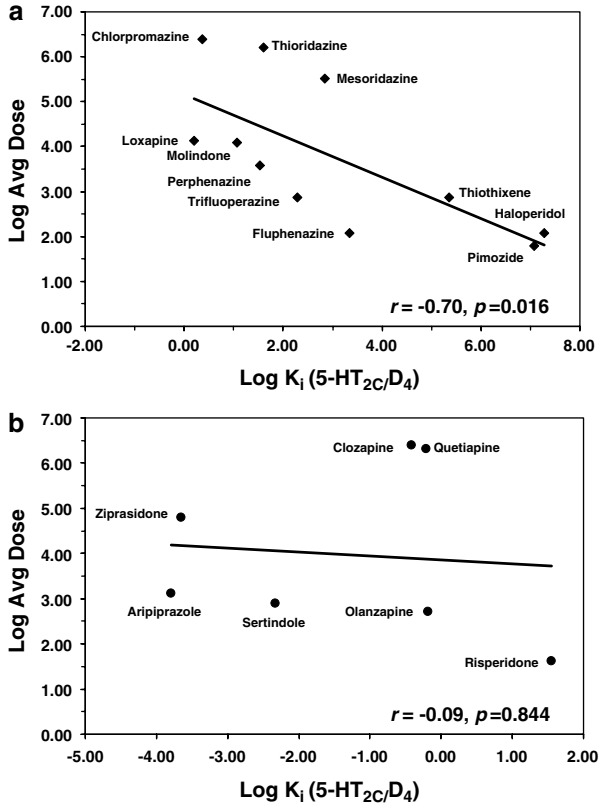

Figure 7 Clinically effective antipsychotic dose *vs* ratio of binding affinity to cloned human serotonin 5-HT_{2C}/DA D_4 receptor for (a) typical and (b) atypical antipsychotic medications.

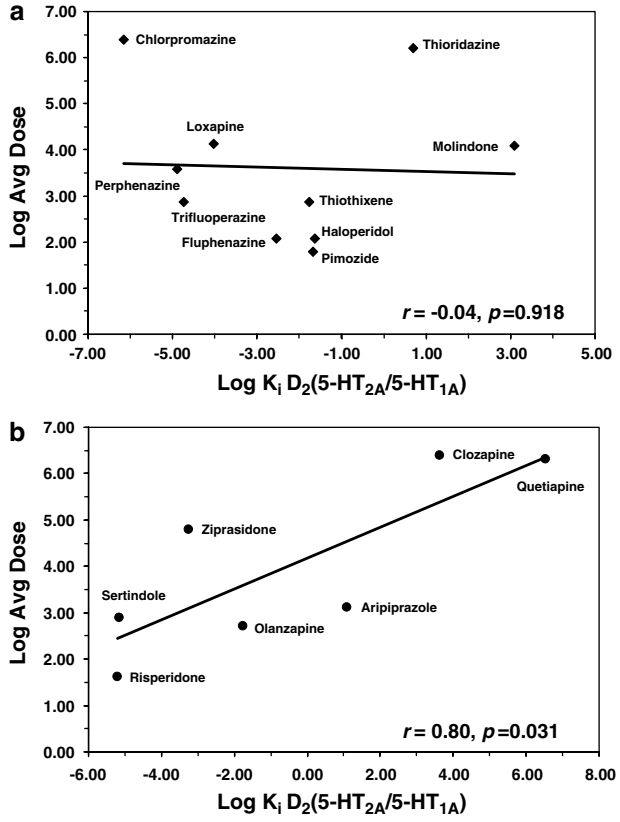

Figure 8 Clinically effective antipsychotic dose *vs* ratio of binding affinity to cloned human D_2 (5-HT_{2A}/5-HT_{1A}) receptor for (a) typical and (b) atypical antipsychotic medications.

the raphe nucleus project diffusely to targets throughout the brain, and strong 5-HT_{2C} receptor expression has been observed in nucleus accumbens and ventral striatum, with modest expression in prefrontal cortex (Lopez-Gimenez *et al*, 2001; Eberle-Wang *et al*, 1997). In prefrontal cortex, 5-HT_{2C} receptors are co-expressed with DA D_4 receptors (Vysokanov *et al*, 1998). Within the substantia nigra/ventral tegmentum, 5-HT_{2C} receptors are expressed on inhibitory GABA-ergic interneurons (Eberle-Wang *et al*, 1997). 5-HT_{2C} receptor stimulation inhibits reward system-related behaviors including cocaine-induced hyperlocomotion (Filip and Cunningham, 2003; Grottick *et al*, 2000). 5-HT_{2C} receptor blockade increases both dopaminergic cell firing and DA release in nucleus accumbens and frontal cortex (Alex *et al*, 2005; Di Matteo *et al*, 1999; Millan *et al*, 1998). 5-HT_{2C} receptor blockade could oppose the actions of D_2 DA receptor blockade either through direct effects on second messenger systems in neurons co-expressing DA D_2 and 5-HT_{2C} receptors, or indirectly through a systems effect on components of limbic neurotransmission. In addition to antagonist effects through inhibition of basal serotonin tone, constitutive activity of 5-HT_{2C} receptor isoforms have also been previously described (Niswender *et al*, 1999; Westphal *et al*, 1995), and this constitutive activity participates in the tonic inhibition of mesolimbic DA function (De Deurwaerdere *et al*, 2004). Antipsychotic medications could therefore also function as inverse agonists through second messenger pathways of 5-HT_{2C} isoforms (Navailles *et al*, 2006; Rauser *et al*, 2001). Although previous studies of the 5-HT_{2C} inverse agonist properties of antipsychotic medications have identified the potential role for inverse agonism in the mechanism of action of antipsychotic efficacy (Navailles *et al*, 2006), our data suggest an alternative possibility that 5-HT_{2C} inverse agonism may also directly *oppose* acute antipsychotic efficacy. Our analysis supports the concept that 5-HT_{2C} agonists, in contrast, may have therapeutic potential as adjunctive medications to improve antipsychotic efficacy for patients receiving typical antipsychotic medication, and suggests that these medications could have applications for treatment refractory psychosis. The potential therapeutic benefit of 5-HT_{2C} stimulation in inhibiting psychotic symptoms through inhibition of meso-accumbens DA function must be balanced with the potential for worsening of cognitive and negative symptoms through decreased mesocortical DA function.

5-HT_{2C}/D_3 and to a lesser extent 5-HT_{2C}/D_4 binding affinity ratios were also correlated with clinically effective antipsychotic medication dose for typical antipsychotic medications. Our data therefore suggest the likelihood of an interaction between binding at serotonin 5-HT_{2C} and DA D_2, D_3, and D_4 receptors in the mechanism of action of typical antipsychotic medications.

Our analysis identifies a modest correlation between antipsychotic drug dosage and the ratio of serotonin 5-HT_{2A}/D_2 receptor affinity for typical antipsychotic medications.

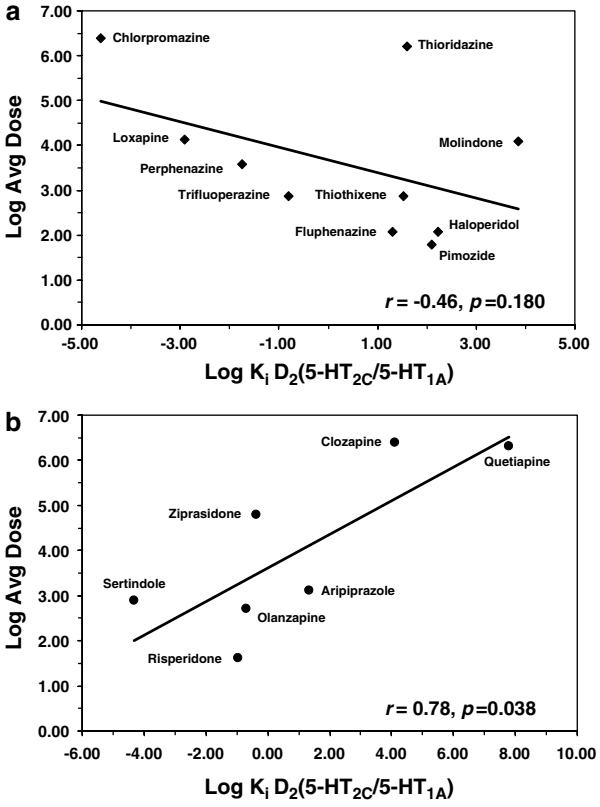

Figure 9 Clinically effective antipsychotic dose *vs* ratio of binding affinity to cloned human D_2 (5-HT_{2C}/5-HT_{1A}) receptor for (a) typical and (b) atypical antipsychotic medications.

Atypical Antipsychotic Medications

Therapeutic efficacy for atypical antipsychotic medications has been suggested to result from a balance of inhibition at DA D_2, serotonin 5-HT_{2A}, and 5-HT_{2C} receptors (Meltzer, 1989, 1995; Leysen *et al*, 1993; Huttunen, 1995), whereas serotonin 5-HT_{1A} receptor stimulation appears to contribute to antipsychotic efficacy in rat models (Protais *et al*, 1994; Meltzer *et al*, 2003; Millan, 2000). Consistent with these observations, clinically effective dosages of atypical antipsychotic medication are highly correlated with the ratios of D_2 (5-HT_{2A}/5-HT_{1A}) and D_2 (5-HT_{2C}/5-HT_{1A}) receptor-binding affinities. Thus, our analysis suggests that the therapeutic effects of atypical antipsychotic medications are determined by interactions among three different domains: (1) increasing D_2 DA receptor-binding affinity enhances antipsychotic potency. (2) Increasing 5-HT_{2C} and 5-HT_{2A} receptor-binding affinities also facilitate antipsychotic efficacy. (3) Increasing 5-HT_{1A} receptor-binding affinity, in contrast, reduces antipsychotic efficacy.

We are not aware of other studies demonstrating that serotonin 5-HT_{2C} receptor blockade has opposite effects in typical and atypical antipsychotic medications. It has previously been suggested, however, that both 5-HT_{2C} antagonism (Meltzer *et al*, 2003; Meltzer, 1995; Wood *et al*, 2006) and 5-HT_{2C} receptor stimulation (Meltzer, 1999; Marquis *et al*, 2007) could facilitate antipsychotic activity. Our data support the view that this seemingly paradoxical finding may result from the relatively higher 5-HT_{2A} receptor blockade in atypical *vs* typical medications. Thus, simultaneous 5-HT_{2A} and 5-HT_{2C} receptor blockade may be more effective in mediating antipsychotic effects than blockade of either receptor separately (Meltzer *et al*, 2003).

Our finding that a simple linear correlation between D_2 receptor binding and clinically effective drug dosage is not apparent for atypical antipsychotic drugs was an unexpected outcome. Previous studies have determined that atypical antipsychotic medications can be distinguished from typical antipsychotic drugs based on the ratio of 5-HT_2/D_2-binding affinities (Meltzer *et al*, 1989a, b). Thus, we had anticipated a more direct relationship between D_2 binding and antipsychotic efficacy for second-generation medications, and an interaction between serotonin 5-HT_{2A} and D_2 effects. Instead, we observed a modest correlation between atypical antipsychotic drug dosage and serotonin 5-HT_{2A} receptor binding, and a similarly modest correlation between DA D_4 receptor-binding affinity and atypical antipsychotic drug dosages. It has previously been suggested that a subset of atypical antipsychotic medications derive their efficacy in part from selective effects at D_4 DA receptors (Seeman *et al*, 1997). Our data do not provide evidence supporting the concept that a simple ratio of binding to 5-HT_{2A} and D_2 receptors accounts for a significant proportion of atypical antipsychotic medication efficacy, although this ratio does appears to distinguish between atypical and typical medication classes (Meltzer *et al*, 1989a, b). Addition of more members of the atypical class to the relatively small number of drugs available for our analysis might help to further clarify this issue.

Limitations

Our analysis is limited to antipsychotic medication effects on positive psychotic symptoms, and does not address efficacy for negative symptoms or cognition, which may be more important in terms of long-term functional outcome. Importantly, the strength of correlations between receptor binding and antipsychotic efficacy identified in our analysis are restricted by a wide range of limiting factors. Medication differences in absorption; metabolism; protein binding; and the presence of pharmacologically active metabolites all serve to weaken the observed correlations. Additionally, the antipsychotic medication dose prescribed to patients may be determined in part by side effects, and might therefore not accurately reflect the 'ideal' efficacy dose. The paucity of adequately powered clinical trials to determine optimal dose for antipsychotic medications further limits the accuracy of medication dosages employed in our analysis. Also, the binding data used in the current analysis, measuring ligand binding to cloned human receptors expressed in cell culture systems, may be distinct from binding to limbic neurotransmitter receptor populations *in vivo*. Differences in receptor phosphorylation, glycosylation, and/or dimerization to hetero-oligomers (Nimchinsky *et al*, 1997; Scarselli *et al*, 2001; Zawarynski *et al*, 1998; Lee *et al*, 2000) between *in vivo* and cell culture systems lacking post-translational machinery could potentially alter receptor-binding affinity. Additionally, atypical antipsychotic medications as a group tend to have more rapid dissociation rates from DA receptors than typical antipsychotics (Kapur and Seeman, 2001), an effect that might further complicate the relationship between receptor affinity and clinically effective drug

dose. And finally, this approach is inherently limited by the complexities of brain circuitry in which DA and serotonin receptors may function as a 'brake' in one brain region, and simultaneously as an 'accelerator' in a different brain region. For example, blockade of D_2 DA autoreceptors in cell body regions of the ventral tegmentum increases both synthesis and release of DA, which could worsen psychotic symptoms, whereas blockade of postsynaptic D_2 receptors in limbic terminal regions would likely have an opposite behavioral effect. Thus, the dysfunction of schizophrenia, resulting from a complex interaction of multiple receptor and neurotransmitter systems (Carlsson *et al*, 1999), does not lend itself ideally to an analysis of isolated receptor systems.

In summary, we present data demonstrating correlations between clinical efficacies of antipsychotic medications and binding affinities to D_2, D_3, D_4, 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptor subtypes. Given the numerous limitations inherent in this approach (listed above), the strength of correlations described in this analysis suggest that the DA and serotonin receptor subtypes analyzed provide the preponderance of antipsychotic effect of these medications. The specific mechanism(s) underlying this clinical effect, however, remains obscure. The 'disconnect' between the pharmacokinetics of receptor blockade and the extended time lag until clinical benefit suggests antipsychotic efficacy, while initiated through binding to neurotransmitter receptor target(s), is likely the result of a downstream cascade of changes in gene transcription and translation. Studies identifying the specific targets of altered gene transcription resulting from these drug-neurotransmitter receptor interactions would therefore have high likelihood of improving specificity and efficacy of antipsychotic medications.

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