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Discriminative Stimulus Properties of Antipsychotics

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GOUDIE, A. J. AND J. A. SMITH. *Discriminative stimulus properties of antipsychotics.* PHARMACOL BIOCHEM BEHAV **64**(2) 193–201, 1999—Drug discrimination methodology has been used in a number of ways to analyze the actions of novel and putative novel antipsychotics in vivo. Recent studies suggest (a) in contrast to earlier theorizing, antagonism of the low-dose *d*-amphetamine stimulus in rats may not be an effective screen for novel antipsychotics; (b) dopamine D_2-like agonists and antagonists, some of which are putative antipsychotics, can be studied in vivo as discriminative cues, although there is a pressing need for more selective drugs that differentiate the various members of the D_2 family; (c) antagonism of the cue induced by the noncompetitive NMDA antagonist MK-801, which has been proposed as a possible screen for clozapine-like compounds, may be an unreliable assay; and (d) the clozapine stimulus is probably a compound cue (a drug "mixture"), which can be used to screen for novel clozapine-like antipsychotics, although the precise receptor mechanisms involved in mediating the clozapine stimulus, and its direct relevance to the antipsychotic action of clozapine remains to be proven conclusively. © 1999 Elsevier Science Inc.

Antipsychotics Neuroleptics Drug discrimination Amphetamine Haloperidol Clozapine Dopamine Serotonin Scopoloamine MK-801

FOLLOWING the development of antipsychotics in the 1950s, a consensus emerged that no particular type of drug was most efficacious in treating schizophrenia (34). Since the publication of a seminal article demonstrating the unique efficacy of clozapine (33), there has been an explosion of research into so-called "atypical antipsychotic" believed to have a number of advantages over older "typical" antipsychotics. Unfortunately, there is no agreed definition of an "atypical" antipsychotic (56). Among the characteristics attributed to such drugs are (a) superior efficacy in treatment resistant patients; (b) reduced extrapyramidal side effects (EPS) compared to typical agents; (c) selectivity of actions at mesolimbic/mesocortical dopamine (DA) systems compared to the nigrostriatal DA system; (d) superior efficacy against negative symptoms and cognitive dysfunction; and (e) minimal elevation of prolactin. However, there is much evidence that novel antipsychotics are an heterogeneous group. Thus, the term "atypical antipsychotic" should probably be avoided (3,34). In this article we, therefore, assume that there are two different classes of antipsychotics—older typical antipsychotics, and novel antipsychotics—which show substantial differences [cf. (3)]. Clozapine remains the "gold standard" for comparisons with typical and novel antipsychotics (36), although the unique mode of action of clozapine is unknown (37), and it remains to be determined whether clozapine is more efficacious clinically than some of the more novel antipsychotics. Clozapine possesses a complex pharmacology, with mainly antagonist, but some agonist, actions at many receptors (4). Thus, it is not surprising that attempts to explain clozapine's unique effects have proved inconclusive. As far as drug discrimination (DD) research is concerned, the rich, "polyvalent" pharmacology of clozapine and many (but not all) novel antipsychotics, coupled with theoretical ideas about the limbic site of action of clozapine, and recent developments in molecular biology have meant that DD methodology has been used in various different ways to characterize the actions of antipsychotics in vivo. It is these different DD assays that we review selectively in this article. We note, however, that, due to limitations of space, this review of relevant DD studies is not exhaustive. There is much evidence that hallucinogenic agents such as LSD and DOM act via serotoninergic systems, and that many novel antipsychotics with potent antagonistic actions on serotonin systems block LSD/DOM discrimination. Indeed, such studies played an important role in the develop-

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ment of risperidone. This important area of DD research has been reviewed recently in detail elsewhere (43), and is considered by Winter and colleagues in this volume.

The Low-Dose Amphetamine Cue as a Putative Screen for Antipsychotics

Antagonist actions at D_2 receptors have been linked for many years to the actions of typical antipsychotics. Such drugs inhibit mesolimbic DA mediated amphetamine-induced hyperactivity. Both typical and novel antipsychotics have preferential effects against low dose (0.5 mg/kg in rats) amphetamine (AMP)-induced hyperactivity (1). Antipsychotic drugs may also have inhibitory actions on nigrostriatal DA systems mediating stimulant-induced stereotypy. Agents selectively inhibiting hyperactivity vs. stereotypy are thought to possess limbic selectivity, and thus be devoid of the EPS induced by blockade of nigrostriatal DA systems. On the basis of such theorising, it was proposed that a low dose (limbically mediated) AMP stimulus (1 mg/kg in rats) could be used to screen for both typical antipsychotics and for novel antipsychotics devoid of EPS (49). This AMP cue was reportedly blocked by both typical agents, and by drugs devoid of EPS (e.g., clozapine and sulpiride). Evidence that the cue properties of AMP are mediated at mesolimbic sites (20,50) supports the hypothesis that antagonism of low-dose AMP discrimination may be of value in screening for antipsychotics. However, recent studies (2) question the value of this screen, because, although the 1.0 mg/kg AMP cue in rats was fully blocked by typical antipsychotics (haloperidol and fluphenazine), novel agents had differential effects. Clozapine and olanzapine blocked the cue substantially (maximum antagonism 77 and 59% respectively), risperidone and remoxipride blocked the cue weakly (maximum antagonism 34 and 24%, respectively), while quetiapine and sertindole had no effects (maximum antagonism 4 and 12%, respectively). Compounds failing to block the AMP cue substantially were studied at doses that reduced responding (sertindole, risperidone, and quetiapine), precluding the testing of higher doses. Furthermore, despite their inability to block the AMP cue, risperidone and sertindole blocked the cue induced by the hallucinogenic 5-$HT_{2A/2C}$ agonist DOI (77 and 84%, respectively), demonstrating their pharmacological activity in vivo. Given that risperidone, remoxipride, sertindole, and quetiapine are all clinically validated antipsychotics, these data question the validity of low-dose AMP discrimination as a screen for antipsychotics. However, in contrast to these findings (2), it has been reported that risperidone fully blocks the cue induced by AMP at 1.25 mg/kg (43). Thus, there is an urgent need to resolve these discrepant findings. With this important caveat, it may be concluded that the low dose AMP cue is probably not a valid screen for antipsychotics. It has been suggested tentatively that, as AMP discrimination is limbically mediated, and associated in humans with euphoria (9), agents that block the AMP cue do so because they arc dysphoric (2). Thus, inhibition of AMP discrimination may be an undesirable property in an antipsychotic. This hypothesis clearly requires further empirical testing before it is accepted.

Dopamine D_2-like Antagonists and Agonists as Discriminative Stimuli

Molecular biological techniques have isolated two families of dopamine receptors, D_1-like receptors, including D_1 and D_5 receptors; and D_2-like receptors, including D_2, D_3, and D_4 receptors (65). We are not concerned here with the D_1 family, as there is no evidence that drugs acting at this receptor are effective antipsychotics; indeed D_1 antagonists may actually exacerbate psychoses (8). However, the D_2 family has consistently been linked with antipsychotic drug actions, and the effects of a number of relatively selective D_2-like agonists and antagonists have been studied with DD methodology. A persistent problem in this area has been the lack of specific agonists and antagonists that differentiate the three members of the D_2-like family (60,62). This situation is complicated further by the fact that drug selectivity actually appears lower in functional assays than in receptor binding tests in vitro (30,38), which may, therefore, be misleading. Indeed, it was suggested recently that "The attribution of in vivo pharmacological effects of (D_3 ligands) to specific receptor subtypes based upon binding data is, in most instances, premature" (62). The actions of drugs from the D_2 family in DD studies up to the end of 1997 have been reviewed elsewhere recently (60).

Dopamine D_2-like antagonists. Given the well-known actions of typical antipsychotics as D_2 antagonists, an obvious strategy for studying such drugs in DD procedures is to train animals to discriminate D_2-like antagonists. Few such studies have been conducted, due to the fact that D_2 antagonists have low discriminability and are difficult to train (25,52). Nevertheless, rats have been trained to discriminate chlorpromazine (18), which generalizes to haloperidol (25). Conversely, rats trained to discriminate haloperidol generalize to chlorpromazine (42), suggesting that typical neuroleptics from different pharmacological classes have similar stimulus properties. In a recent important study, which may resurrect interest in this area, the cue induced by tiapride, a benzamide with $D_{2/3}$ antagonist actions and limited ability to induce sedation and catalepsy, was analyzed (17). Rats discriminated a dose of tiapride that suppressed responding by circa 50%, although only after extensive training. A series of benzamides (clebopride, sultopride, sulpiride, amisulpride, raclopride, and remoxipride) all generalized by 80% or more to tiapride at rate-suppressant doses. Dose-related substitution (75–100%) was also induced by various nonbenzamide DA antagonists: chlorpromazine, risperidone, haloperidol, pimozide, thioridazine, and olanzapine. Intriguingly, clozapine produced no generalization, even at doses that markedly suppressed responding. The tiapride cue was antagonized by the nonspecific DA releaser AMP, and by the D_2/D_3 agonists 7-OHDPAT and quinpirole, but not by the D_1 agonist SKF 38393, suggesting, that the cue is mediated by antagonist actions at D_2/D_3 receptors. These results accord with evidence that selective $D_{2/3}$ antagonists, like haloperidol and chlorpromazine, are difficult to train. Nevertheless, such drugs clearly can be studied in DD assays. Tlc generalization to tiapride seen with the novel antipsychotics risperidone and olanzapine, coupled with the absence of such generalization with clozapine, shows that novel antipsychotics can be differentiated in DD assays, demonstrating the heterogeneity of such drugs. The results with olanzapine are particularly intriguing, as olanzapine is a clozapine congener with many pharmacological actions similar to clozapine (45), and which substantially generalizes to clozapine in some studies [e.g, (53)]. However, olanzapine has higher affinity than clozapine for the D_2 receptor (13), which presumably accounts for the different patterns of generalization to tiapride seen with the two drugs. In summary, these findings show that D_2/D_3 antagonists can be studied in DD assays, allowing valuable insights into the receptor mechanisms involved in various drugs' actions, and important differences to be demonstrated between novel antipsychotics.

Specific dopamine D_3 antagonists. The D_3 receptor has attracted much interest as a possible target for antipsychotics, as many antipsychotics show high D_3 affinity. Furthermore, the D_3 receptor is located mainly in the limbic system, in areas associated with cognition and affect, which are disturbed in psychoses (30,64). D_2 and D_3 antagonists appear to have opposite effects on locomotor activity, the former being inhibitory, and the latter excitatory (62), leading to the suggestion that D_2-mediated actions may be associated with EPS, and D_3-mediated actions with alleviation of negative symptoms, and that specific D_3 antagonists may be antipsychotics devoid of motor side effects and perhaps with greater efficacy than D_2 antagonists (62).

The putative D_3 antagonist PNU-99194A is circa 20-fold selective for D_3 vs. D_2 receptors, and does not bind (K_is > 1000 nM) to other monoaminergic, cholinergic, or opioid receptors (73). PNU-99194A resembles other D_3 antagonists in stimulating locomotor activity (73). PNU-99194A induces a discriminative stimulus in rats, which, despite the drug's locomotor stimulant properties, differs from the stimulus induced by cocaine and amphetamine (6). In a recent study (21) the PNU-99194A stimulus was attributed to selective D_3 antagonism, because it generalized fully to the D_3 preferring antagonists (−)-DS121 and (+)-AJ76, while nonselective D_2 antagonists such as haloperidol failed to substitute. Various different types of DA agonists (direct, indirect, and D_1 and D_2 selective) failed to generalize PNU-99194A. The conclusion that D_3 antagonism mediates the PNU-99194A cue obviously presupposes that the drug is a selective D_3 antagonist. Recently the ethopharmacological behavioral profiles of three putative selective D_3 antagonists (PNU-99194A, GR 103691, and nafadotride) in rats were described (16). A difference in the mechanisms of action of PNU-99194A in vivo compared to GR 103691 and nafadotride was hypothesized, because the profile of PNU-99194A only was similar to that of both D_2-like and D_1-like agonists (16). However, even if PNU-99194A does possess such D_1- and D_2-like agonist actions in vivo, they would not appear to be relevant to the PNU-99194A cue, because neither D_1 nor D_2 agonists substituted for PNU-99194A (21). Nevertheless, such findings (16) do suggest caution in unequivocally attributing the PNU-99194A cue to D_3 antagonism. Further studies are clearly required with more selective tools, if and when they become available.

Specific dopamine D_3 agonists. Recent studies indicate that purported selective D_3 agonists are discriminable, following an initial report (41) suggesting that the cue induced in rats by 7-OHDPAT, a preferential D_3 agonist (39) might be mediated by actions at D_3 receptors. However, as with the antagonist studies described above, problems arise from the limited selectivities of training and test drugs. In an attempt to resolve the issue of whether specific cues are mediated by actions at either D_2 or D_3 receptors, in some studies the ability of a number of agonists with varying D_2 and D_3 selectivities to generalize to the relevant training drug have been determined, and then correlations between the generalization ED_{50}s with the potencies of the test drugs in functional assays for either D_3 or D_2 receptors (specifically DA stimulated mitogenesis in cell lines expressing either receptor) have been calculated. In one such study (58) rats were trained to discriminate 7-OHDPAT. The DA agonists quinelorane, quinpirole, apomorphine, PD 128,907, bromocriptine, as well as 7-OHDPAT itself all induced substantial dose-related generation. When generalization ED_{50}s were correlated with functional D_2- and D_3-mediated actions, [see (61)], the correlation with D_3 discriminative actions (+0.98) was significant, but that with D_2 actions (−0.16) was not, suggesting that the 7-OHDPAT cue was mediated at the D_3 receptor. However, the authors were appropriately cautious in interpreting their data (58), pointing out that, if the data obtained with bromocriptine (the only drug studied with greater D_2 than D_3 affinity) were deleted from the analysis, then the significant correlation with D_3 affinity simply "disappeared." Similar results from a different laboratory were obtained in other rats also discriminating 7-OHDPAT (72). The mixed DA agonists quinelorane, quinpirole, apomorphine, PD 128,907, and 7-OHDPAT all induced full dose-related generation. When generalization ED_{50}s were correlated with functional D_2 and D_3-mediated effects (61), the correlation with D_3 discriminative actions (+0.99) was significant, but that with D_2 actions (+0.23), was not, again suggesting that the 7-OHDPAT cue is mediated by actions at the D_3 receptor (72). However, these authors were also cautious in their conclusions, arguing that studies with more selective agents are required in this area.

The actions of the preferential D_3 agonist (+)-PD 128,907 (55), in generalizing to the cue induced by the nonspecific DA agonist apomorphine, have also been studied (38). PD 128,907 generalized fully, and this effect was antagonized by the D_3 preferring antagonists (+)-AJ76 and (+)-UH 232, and by the D_2 antagonist haloperidol. However, haloperidol was 30–130 times more potent than the D_3 antagonists in blocking PD 128,907 generalization. Because this ratio approximated more closely to the in vitro D_2 binding affinities of these drugs relative to haloperidol than to their D_3 affinities relative to haloperidol, the authors concluded that the ability of PD 128,907 to generalize to apomorphine was probably mediated at D_2 not D_3 receptors, although they also emphasized the need for studies with more selective agents (38). In direct contrast, using the type of functional correlational analysis outlined above, other authors (58) have concluded that the apomorphine cue is mediated at D_3 not D_2 receptors, because the functional correlation with D_3 discriminative actions (+0.98) was significant, but that with D_2 actions (−0.13) was not. Thus, completely opposite conclusions were drawn about the role of D_3 receptors in mediating the stimulus properties of apomorphine (38,58), showing graphically that it is very difficult, if not impossible, to make definitive inferences about the role of D_3 receptors in specific drug stimuli, because the conclusions drawn depend critically upon the specific drugs studied and the precise type of analyses conducted on, and inferences drawn from, generalization data.

In the studies with PD 128,907 outlined above, the authors concluded cautiously that their generalization data did not preclude the possibility that PD 128,907 might act via D_3 receptors when used as a training, rather than a test, drug (38). However, PD 128,907 has more recently been studied as a training drug (10), and it was concluded that the cue was probably D_2 mediated. Various nonselective $D_{2/3}$ agonists generalized fully to PD 128,907 (apomorphine, quinpirole, and 7-OHDPAT); and nonselective $D_{2/3}$ antagonists (haloperidol, raclopride, and spiperone) attenuated the PD 128,907 cue. However, selective D_3 antagonists (GR 103,691 and L-745,829) did not block the cue. In contrast, the selective D_2 antagonist L741,626 did, leading the authors to conclude, that it was D_2 mediated, and thus that PD 128,907 was not a selective D_3 agonist in vivo (10), in agreement with the earlier conclusions based upon generaliation to apomorphine (38).

Collectively, the data reported from DD analyses of agonists and antagonists from the D_2 family obviously demonstrate the need for cautious interpretation of data when drawing inferences about receptor-mediated stimuli, as stressed by others (62). They also highlight the need for more selective drugs. Although almost all research into D_3 ligands has been

based on the premise that D_3 antagonists may be effective antipsychotics, there are suggestions that D_3 agonists may be effective antipsychotics (75), perhaps by acting presynaptically inhibiting DA release (32).

MK-801 Discrimination as a Selective Screen for Clozapine-Like Antipsychotics

It has been reported (19) that, in rats discriminating the noncompetitive NMDA antagonist MK-801 at 0.075 mg/kg from saline in a discrete-trial shock-avoidance paradigm, clozapine, but not haloperidol, blocked the MK-801 cue. Haloperidol was tested up to the highest dose at which rats would respond, but failed to attenuate the MK-801 cue even partially. In direct contrast, clozapine produced full dose-related antagonism of the cue. These data were taken to suggest that the MK-801 cue may be used to selectively screen for clozapine-like antipsychotics (19). Given that noncompetitive NMDA antagonists such as PCP and MK-801 are thought to induce a "model" of psychosis, which, unlike amphetamine, mimics both positive and negative symptoms (70), and evidence that antipsychotics can block various different effects of noncompetitive NMDA antagonists [e.g. (7,24,40,57)], this idea has considerable intuitive appeal, as advocated in a major recent review of novel antipsychotics (3). Nevertheless, in extensive studies on the MK-801 cue in a food rewarded task (see accompanying empirical article by Smith and Goudie in this journal), we found that rats trained to discriminate MK-801 at 0.075 mg/kg [the training dose used in (19)] showed no evidence of antagonism with clozapine at doses up to the highest that could be tested [6 mg/kg—twice the dose reported (19) to completely block the MK-801 cue]. Thus, it is unclear whether the MK-801 cue can actually be used to selectively screen for novel clozapine-like antipsychotics. It is possible that the MK-801 cue differs in shock avoidance and food-rewarded paradigms. However, until this has been shown convincingly, MK-801 discrimination should, therefore, probably not be considered a reliable screen for clozapine-like novel antipsychotics at present. Furthermore, if the MK-801 cue does actually turn out to be a reliable screen for clozapine-like novel antipsychotics, albeit only under certain specific conditions, it will be necessary to show that both PCP and ketamine, which have been studied extensively in DD tasks, and which are both psychotomimetic noncompetitive NMDA antagonists, are both also reliable DD screens for clozapine-like novel antipsychotics under the same conditions. (See the article by Koek and colleagues in this volume for a fuller discussion of the cue induced by NMDA antagonists).

The Clozapine Cue

Although the older typical antipsychotics are difficult to train, it has been known for some years that clozapine is more readily discriminable, and that typical antipsychotics do not generalize to clozapine (11,25). Following these early studies, clozapine DD has been studied extensively in rats (22,27,29, 35,44,45,48,51,54,71,74), pigeons (31), and monkeys (15). Three major conclusions have arisen from this work. First, the clozapine cue is probably a compound cue, requiring concurrent actions at various different receptors. Second, the specific complex of receptors mediating the clozapine cue is unknown; and third, the relationship, if any, between the antipsychotic actions of clozapine and the drug's discriminative actions is also unknown. These three issues will be considered in turn.

The evidence that the clozapine cue is a compound cue derived largely from comparisons of studies with receptor specific ligands and agents with actions at many different receptors. Typically, specific receptor ligands for a very wide range of receptors do not generalize fully (75% or more) in a range of species to clozapine, including antagonists acting at all of the following receptors: dopamine D_1 (22,27,31); dopamine D_2 (11,15,22,25,29,31,45,51,54,71,74); dopamine D_4 (27); alpha-noradrenergic$_1$ (22,27); alpha-noradrenergic$_2$ (22,27); histamine$_1$ (27); 5-HT_{2A} (27,31,48,71); 5-HT_{2C} (27,74); and 5-HT_3 (27,74). Furthermore 5-HT_{1A} agonists do not fully generalize to clozapine (22,27,31,74), and 5-HT_{1A} antagonists do not block the clozapine cue (27), despite evidence that clozapine may be a 5-HT_{1A} agonist (47). It is also not possible to block the clozapine cue with an antagonist at D_1 receptors (27), despite some evidence that clozapine may be a D_1 agonist (4).

In contrast to the results obtained with the receptor-specific ligands described above, various drugs with affinities for many different receptors have been reliably shown to fully generalize to clozapine, including cyproheptadine [(11,31); Goudie and Smith, unpublished], the clozapine congeners olanzapine (45), quetiapine (14,29), zotepine (Smith et al., unpublished); JL13 (12,29), JL 5, JL 8, and JL 18 (14,15), perlapine, and fluperlapine (14,15), and the "multireceptor" putative antipsychotics PNU 96415 (27,71), and S16924 (44). It has also been reported that rats trained to discriminate olanzapine fully generalize to clozapine (53). When systematic studies of various antipsychotics have been run in clozapine-trained rats (29,54) and monkeys (14,15), it has been found that typical antipsychotics do not generalize; nor does amisulpiride, a selective $D_{2/3}$ antagonist antipsychotic; neither do agents such as risperidone and sertindole that have affinity for D_2 5-HT_{2A}, and alpha$_1$ receptors; while agents with broader profiles of receptor binding such as chlorpromazine and thioridazine tend to generalize only partially. However, antipsychotics with very broad ranges of receptor binding fully generalize, as described above. Figures 1–4 show representative data illustrating this point, from rats trained to discriminative clozapine at 5 mg/kg (29). Such rats showed dose-related generalization to clozapine, as expected (Fig. 1); typical neuroleptics such as loxapine and haloperidol did not generalize at doses with marked-rate suppressant actions (Fig. 2); only two multireceptor antagonists, JL13 and seroquel (quetiapine), fully generalized (Fig. 3); the apparently surprising failure of the multireceptor antagonist olanzapine to fully generalize is attributed to its relatively high D_2 affinity (29) [see also Carey and Bergman (15)]. Antipsychotics such as risperidone, sertindole, and amisulpride with more restricted profiles of receptor actions than clozapine did not fully generalize at doses with marked rate suppressant actions (Fig. 4). Thus, these data clearly show that most generalization to clozapine is seen with antipsychotics with concurrent actions at many receptors. Similar observations have led a number of other authors to conclude independently that the clozapine cue is a compound cue (15,44,71).

However, the notion that the clozapine cue is a compound cue encounters one major difficulty in that the sole purported selective receptor ligand, which has consistently been reported to fully generalize to clozapine, is the muscarinic antagonist scopolamine (27,35,45,48). Indeed, evidence has been provided that muscarinic$_1$, but not muscarinic$_2$ antagonists, fully generalize to clozapine (35). The suggestion that the clozapine cue is mediated solely by muscarinic antagonist actions is, however, paradoxical; because a number of the multireceptor antagonists that fully generalize to clozapine have

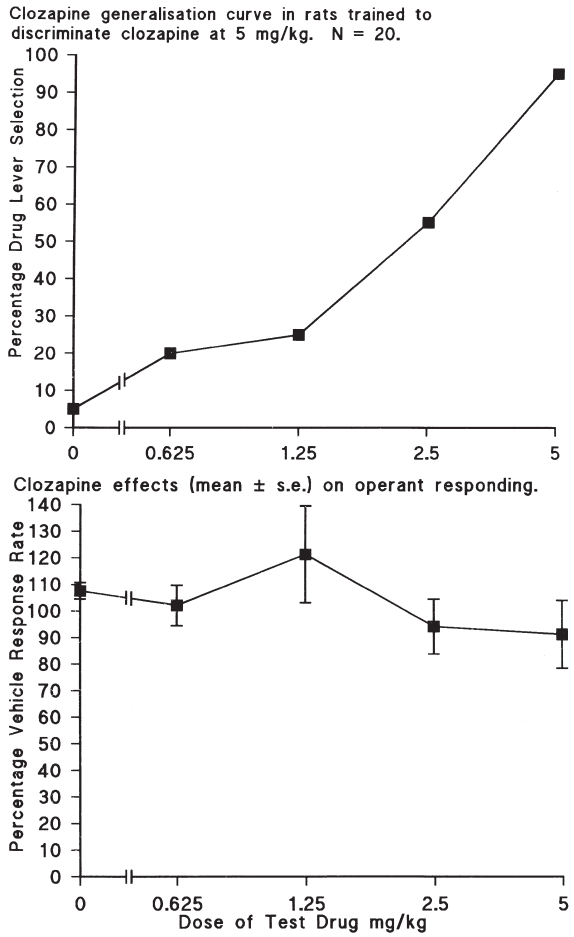

FIG. 1. All figures reproduced from Goudie and Taylor (29) with permission. (Top panel) generalization data for clozapine. All tests involved 20 rats. (Lower panel) Effects of clozapine on response rate expressed as mean (±SE) of most recent vehicle training session.

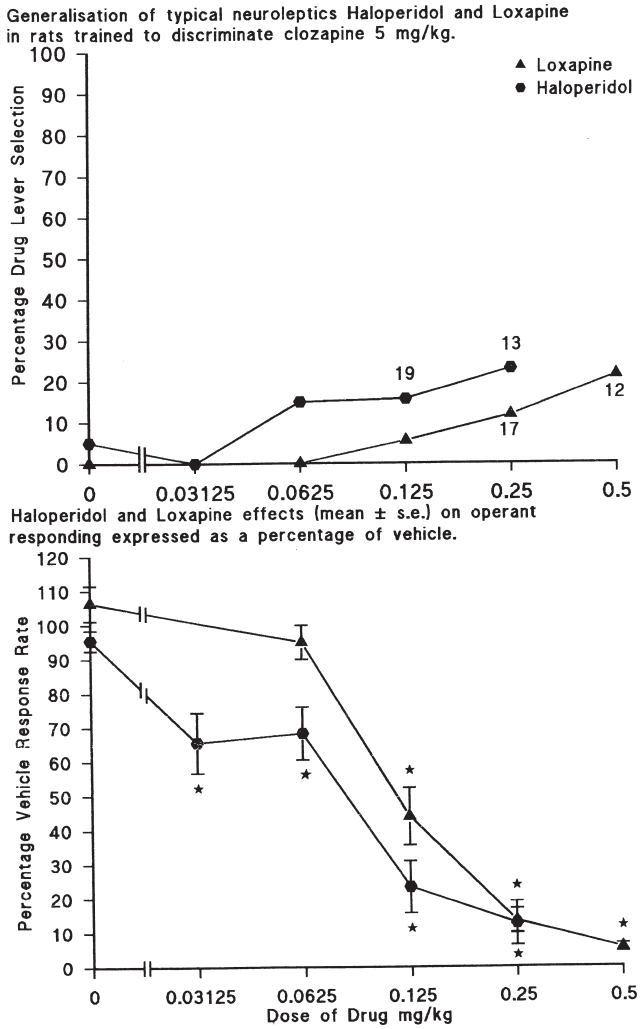

FIG. 2. (Top panel) Generalization data for haloperidol and loxapine. All tests involved 20 rats. Numbers above specific points represent the size of the subset of the total group that actually made a lever selection. For example, at the 0.125 mg/kg dose of haloperidol only 19 out of 20 rats made a lever selection. (Lower panel) Drug effects on response rate expressed as mean (±SE) of most recent vehicle training session. Asterisked points differed significantly from the appropriate vehicle control.

negligible muscarinic affinity, including JL 13 (12), quetiapine (26), zotepine (46), and PNU 96415 (71). Thus, muscarinic affinity appears to be a sufficient, but not a necessary condition, for full generalization to clozapine. There are at least four possible explanations for this apparent paradox. Firstly, it may simply be the case that the doses of scopolamine (and other muscarinic antagonists) that fully generalize to clozapine do not actually have specific actions on muscarinic systems. This issue probably merits further study. Second, M_1 antagonists may have unspecified effects downstream from the M_1 receptor that are similar to those of clozapine, although this hypothesis is essentially untestable, as the specific "effects" are obviously unspecified. Third, if the clozapine cue is a compound cue, it is essentially a drug mixture, as studied in detail in recent years by Stolerman, Gauvin, and colleagues (23,67–69). Perhaps counterintuitively, such studies have typically shown that individual components of drug mixtures are usually processed independently, such that if a discrimination is based on a mixture of Drugs A and B, which have equal salience, if either A or B are given alone at a dose higher than that in the training mixture they induce full generalization to the mixture. Thus, if the compound clozapine cue is functionally equivalent to a "mixture," a high enough dose of a drug acting at any receptor involved in the "mixture" should induce full generalization if, and only if, such doses can be tested without suppressing responding. Thus, full generalization seen with scopolamine may simply reflect that fact that this specific drug can be tested at high enough doses to fully generalize to the clozapine "mixture," while drugs acting at other receptors that are also involved in the "mixture," possibly the $alpha_1$-adrenoreceptor because $alpha_1$ antagonists have consistently been found to partially generalize to clozapine (27), cannot actually be tested at high enough doses to induce full generalization. A fourth, related explanation for the paradox that muscarinic antagonists fully generalize to clozapine when drugs with minimal muscarinic affinity also fully generalize assumes that, in the absence of any muscarinic

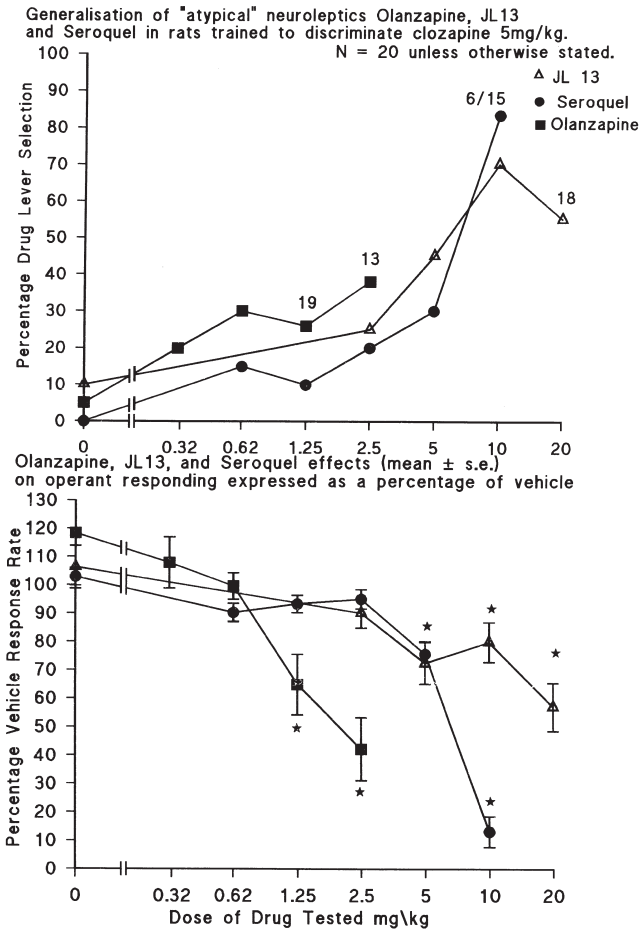

FIG. 3. (Top panel) Generalization data for olanzapine, seroquel (quetiapine), and JL 13. All tests involved 20 rats excepted that at the highest does of seroquel, at which only 15 rats were tested. Numbers above the points represent the size of the subset of the total group that actually made a lever selection. (Lower panel) Drug effects on response rate expressed as mean (±SE) of the most recent vehicle training session. Asterisked points differed significantly from the appropriate vehicle control.

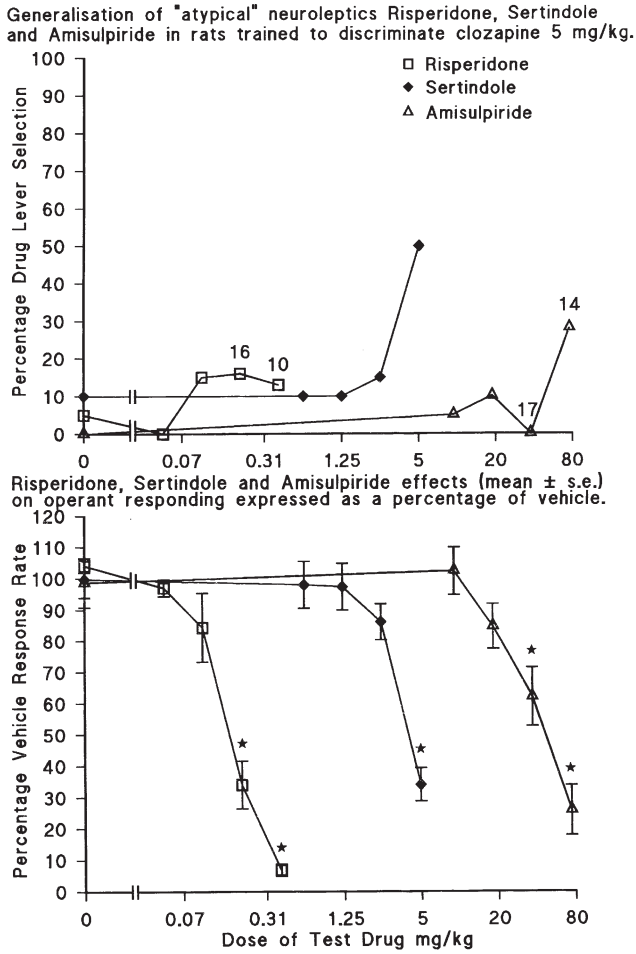

FIG. 4. (Top panel) Generalization data for risperidone, sertindole, and amisulpride. AR tests involved 20 rats. Numbers above specific points represent the size of the subset of the total group that actually made a lever selection. (Lower panel) Drug effects on response rate expressed as mean (±SE) of the most recent vehicle training session. Asterisked points differ significantly from the appropriate vehicle control.

effects, other receptor-mediated stimuli processed in parallel independently to the "muscarinic stimulus" may have additive actions, and thus fully generalize when no one single stimulus does. Thus, drugs with "polyvalent" pharmacology may fully generalize to clozapine by additive, or perhaps even supra-additive actions, at various different receptors. Further extensive studies with drug mixtures will be required to address this specific hypothesis, and determine whether two, three, or fourfold drug mixtures in various combination can induce the clozapine cue by either additive or supra-additive actions. However, the number of drug mixtures that could be studied at different dose combinations in such studies is clearly formidable!

A fundamental issue that needs to be considered when discussing the clozapine cue is whether it is in any way related to the antipsychotic actions of clozapine. Because the mode of action of clozapine as an antipsychotic is unknown, it is very difficult to prove the hypothesis that clozapine discrimination is an assay that is unequivocally relevant to clozapine's antipsychotic actions. Taken at face value, the data obtained with scopolamine could obviously be interpreted as an important "false positive" in this regard, because muscarinic antagonists are not typically regarded as antipsychotics. However, if the doses of scopolamine that generalize to clozapine have many of the same neurochemical actions as clozapine, then it would obviously be possible to account for this apparent "false positive." A novel, but speculative, way of trying to validate clozapine discrimination as an assay that is related to clozapine's antipsychotic actions is to consider the actions of the multireceptor antagonist cyproheptadine, which has consistently been found to generalize to clozapine (11,31). In our studies in clozapine-trained rats full clozapine generalization occurred in the absence of any response rate suppression (27,29), due to the development of tolerance to the well-known rate-suppressant actions of clozapine (59). In contrast, agents such as quetiapine, JL 13 and zotepine, which fully generalized to clozapine, only did so at rate-suppressant doses [(28,29); see also (15)], so they do not fully mimic the actions of clozapine in the DD assay. In contrast, cyproheptadine,

fully generalized in the absence of response-rate suppression [(11); unpublished studies of Goudie and Smith]. Thus, cyproheptadine mimics clozapine fully in the DD assay and to a greater extent than most novel antipsychotics. Cyproheptadine is a nonspecific 5-HT and H_1 antagonist with concurrent actions at muscarinic and alpha$_1$ adrenoreceptors. Some very preliminary clinical data suggest that cyproheptadine may alleviate cognitive dysfunction and negative symptoms of schizophrenia (63), as well, as inhibiting neuroleptic-induced akathisia (5). Clearly, the results of clozapine DD studies provocatively suggest that cyproheptadine should be a clozapine-like antipsychotic, if and only if, clozapine discrimination is related to clozapine's antipsychotic actions. If it is not, cyproheptadine (possibly like scopolamine) would represent an important "false positive," and strongly suggest that clozapine discrimination is not necessarily related to clozapine's antipsychotic actions. Because it has been suggested that the selective 5-HT_{2A} antagonist M100907 may be an effective antipsychotic without any actions on DA systems (66), if clinical trials with M1009907 prove positive, it would be of interest to assess whether cyproheptadine is also an effective antipsychotic in the absence of actions on DA systems, as the clozapine discrimination data suggest it should be. At present, it is clear that both typical and novel antipsychotics may be clinically efficacious without generalizing to clozapine. Generalization to clozapine may, however, indicate that a specific drug is a clozapine-like antipsychotic, although this conclusion must remain tentative at present in the absence of critical clinical data with agents such as cyproheptadine.

In summary, various different types of DD studies have been of considerable value in the study of the actions of both typical and novel antipsychotics in vivo. Refinements of such procedures in the future should ensure that they will continue to play an important role in antipsychotic drug development.

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