A comparison of drug effects in latent inhibition and the forced swim test differentiates between the typical antipsychotic haloperidol, the atypical antipsychotics clozapine and olanzapine, and the antidepressants imipramine and paroxetine

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Current animal models of antipsychotic activity that have the capacity to dissociate between typical and atypical antipsychotic drugs (APDs) have two drawbacks: they require previous administration of a psychotomimetic drug, and they achieve the dissociation by demonstrating effectiveness of atypical but not typical APDs, thus losing specificity and selectivity for APDs. The present experiments were designed to solve these problems by using two non-pharmacological tests: latent inhibition (LI), in which potentiation of the deleterious effects of non-reinforced stimulus pre-exposure on its subsequent conditioning served as a behavioral index for a common action of typical and atypical APDs (antipsychotic), and the forced swim test (FST), in which reduction of immobility served as a behavioral index for a dissimilar action of these drugs (antidepressant). The typical APD haloperidol (0.1 mg/kg), the atypical APDs clozapine (2.5 mg/kg) and olanzapine (0.6 mg/kg), and the antidepressants imipramine (10 mg/kg) and paroxetine (7.0 mg/kg), produced distinct patterns of action in the two tests: haloperidol potentiated LI and increased immobility in the FST, clozapine and olanzapine potentiated LI and decreased immobility in the FST, and imipramine and paroxetine decreased immobility in the FST and did not potentiate LI. Thus, the comparison of drug effects in LI and FST enabled a discrimination between typical and atypical APDs without losing selectivity for APDs.


Keywords: antipsychotic drugs, antidepressant drugs, latent inhibition, forced swim test, rat

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Introduction

Antipsychotic drugs (APDs) are divided into two groups, typical and atypical. There are several criteria for this distinction, reviewed extensively elsewhere (Brunello et al., 1995; Kinon and Lieberman, 1996; Arnt and Skarsfeldt, 1998); the most accepted criteria of atypicality in the clinic are superior therapeutic efficacy and reduced capacity to cause extrapyramidal side-effects. A major challenge faced by animal models of antipsychotic activity is to enable dissociation between typical and atypical APDs. While such models exist, notably, prepulse inhibition (e.g. Bakshi and Geyer, 1995; Swerdlow et al., 1996), social isolation (Sams-Dodd, 1996, 1997), and the forced swim test (FST; Noda et al., 1995), they have two shortcomings. First, all of them require previous administration of a psychotomimetic drug (PCP or MK-801) for demonstrating the dissociation, and therefore are likely to reveal only antipsychotic action that is mediated via neurotransmitter systems affected by the challenge drug. Secondly, these models achieve the dissociation by demonstrating effectiveness of atypical versus inefficacy of typical APDs so that the dissociation entails, rather paradoxically, loss of specificity and selectivity for APDs (Weiner et al., 2000).

Behavioral models that can dissociate between typical and atypical APDs, while preserving their selectivity for APDs, must be able to detect both a common (‘typical’) and a different (‘atypical’) action of these drugs, i.e. provide distinct behavioral indices for each of the actions. In other words, such models should reveal specific patterns of behavioral drug action rather than single behavioral effects. In addition, it would be desirable that such models were non-pharmacological, i.e. did not require the administration of a psychotomimetic drug in order to index both the shared and the different actions of APDs. The present study sought to achieve this aim by using two non-pharmacological tests, namely, latent inhibition (LI), for detecting a common action of typical and atypical APDs (antipsychotic), and the FST, for detecting an action on which the two classes of drugs apparently differ (antidepressant).
In LI, retarded conditioning to a previously non-reinforced stimulus is considered to reflect the capacity to ignore irrelevant stimuli, and therefore to model a cognitive process that is impaired in schizophrenia (e.g. Weiner, 1990, 2003; Gray et al., 1991; Moser et al., 2000; Weiner et al., 2000). Potentiation of LI under conditions that do not yield LI in controls (low number of pre-exposures or extended conditioning) is a well-established behavioral index of antipsychotic activity, obtained with a variety of typical and atypical APDs differing in their in vivo and in vitro pharmacology (for reviews, see Moser et al., 2000; Weiner et al., 2000).

In the FST, immobility has been considered to reflect a state of ‘despair’ in the rat, and reduction in immobility serves as a specific and selective index of antidepressant activity (e.g. Porsolt et al., 1977, 1978; Detke et al., 1995; Lucki, 1997; Bourin et al., 1998; Page et al., 1999). Our choice of the FST was based on the mounting evidence that atypical APDs, such as clozapine, risperidone and olanzapine, have antidepressant activity (Hillert et al., 1992; Tøllefsen et al., 1998; Tøllefsen and Sanger, 1999; but see Abraham et al., 1997), whereas typical APDs induce a variety of depressive symptoms in schizophrenic patients (Harrow et al., 1994; Heinz et al., 1994, 1998). This suggests that antidepressant-like action may also distinguish atypical from typical APDs in animal models, and there is indeed some evidence that such a dissociation in the FST can be obtained without previous drug administration. Thus, whereas the typical APD haloperidol increases immobility (Borsini et al., 1984; Kawashima et al., 1986), the atypical APD clozapine decreases immobility or has no effect (Browne, 1979; Borsini et al., 1984; Gorka and Janus, 1985; Kawashima et al., 1986).

We expected that typical APDs, atypical APDs and antidepressants would produce distinct patterns of action in the LI and the FSTs which would discriminate between typical and atypical APDs, as well as between APDs and antidepressants. More specifically, we expected that typical APDs would increase immobility in the FST and potentiate LI; atypical APDs would decrease immobility in the FST and potentiate LI; and antidepressants would decrease immobility in the FST and not potentiate LI. We first tested whether this pattern of results would be obtained with a representative drug from each class, namely, the typical APD haloperidol (0.1 mg/kg), the atypical APD clozapine (2.5 mg/kg) and the classical tricyclic antidepressant imipramine (10 mg/kg) (Experiments 1–4). The doses were chosen on the basis of our previous work with LI and the relevant literature on the effects of these drugs on FST and LI (Gorka and Janus, 1985; Dunn et al., 1993; Moran et al., 1996; Hitchcock et al., 1997; Weiner et al., 1997; Shadach et al., 2000). After obtaining the predicted pattern of results with the above compounds, we tested an additional atypical APD, olanzapine (0.6 mg/kg) and an additional antidepressant, the selective serotonin reuptake inhibitor (SSRI) paroxetine (7 mg/kg), neither of which had been examined in the non-standard test (Experiments 5 and 6). The doses were chosen on the basis of the relevant literature (Gosselin et al., 1996; Redrobe et al., 1998) as well as pilot studies of olanzapine in LI and paroxetine in FST. Since LI potentiation by APDs is manifested under conditions that do not produce LI in control animals, we used parameters of pre-exposure and conditioning that do not yield LI in control rats (40 pre-exposures and five conditioning trials; Shadach et al., 1999, 2000).

Methods
Subjects
Male Wistar rats (Tel Aviv University Medical School, Israel), approximately 4 months old and weighing 290–400 g, were housed four to a cage under reversed-cycle lighting (lights on: 19.00–07.00 hours) with free access to food and water, except for the duration of the LI experiments. All experimental protocols were carried out according to the guidelines of the Institutional Animal Care and Use Committee of Tel Aviv University.

Apparatus and procedure
Latent inhibition
Rats were tested in Campden Instruments rodent test chambers with a retractable bottle. When the bottle was not present, the hole was covered by a metal lid. Licks were detected by a Campden Instruments drinkometer. The pre-exposed to-be-conditioned stimulus was a 10 s, 80 dB, 2.8 kHz tone produced by a Sonalert module. Shock was supplied through the floor by a Campden Instruments shock generator and shock scrambler set at 0.5 mA and 1 s duration. Equipment programming and data recording were computer controlled.

Prior to the beginning of each LI experiment, rats were handled for about 2 min daily for 5 days. A 23 h water restriction schedule was initiated simultaneously with handling and continued throughout the experiment. On the next 5 days, rats were trained to drink in the experimental chamber for 20 min/day. Water in the test apparatus was given in addition to the daily ration of 1 h given in the home cages. The LI procedure was conducted on days 11–14 and consisted of the following stages given 24 h apart.

Pre-exposure. With the bottle removed, the pre-exposed (PE) rats received 40 tone presentations with an inter-stimulus interval of 50 s. The non-preexposed (NPE) rats were confined to the chamber for an identical period of time without receiving the tone.

Conditioning. With the bottle removed, each rat received five tone–shock pairings given 5 min apart. Shock
followed tone termination immediately. The first tone–
shock pairing was given 5 min after the start of the
session. After the last pairing, rats were left in the
experimental chamber for an additional 5 min.

Retraining. Rats were given a 15 min drinking session as
in initial training. Data of rats that failed to complete 600
licks were dropped from the analysis.

Test. Each rat was placed in the chamber and allowed
to drink from the bottle. When the rat completed
75 licks, the tone was presented for 5 min. The following
times were recorded: time to first lick, time to complete
licks 1–50, time to complete licks 51–75 (before
tone onset; A period) and time to complete licks
76–100 (after tone onset; B period). The amount of
suppression of licking was measured using a suppression
ratio A/(A + B). LI is manifested by lower suppression of
drinking (higher suppression ratios) in PE compared to
the NPE rats.

Forced swim test
Each rat was forced to swim for 15 min inside a vertical
Plexiglas cylinder (height, 40 cm; diameter, 20 cm)
containing 20 cm of water maintained at 27°C, and was
then taken out and allowed to dry for 30 min in a heated
room before being returned to its home cage. Twenty-
four hours later, the rat was replaced into the cylinder and
the total duration of immobility was measured during a
5 min test. The rat was judged to be immobile whenever
it remained floating passively in the water in a slightly
hunched but upright position, its head just above the
water surface (Porsolt et al., 1978).

Drugs
Each of the drugs was administered i.p. in a volume of
1 ml/kg, prior to the pre-exposure and conditioning stages
in LI and prior to the test session in FST. Haloperidol,
prepared from an ampoule containing 5 mg haloperidol in
1 ml solvent containing 6 mg lactic acid (Janssen,
Belgium) and diluted with saline, was administered
60 min prior to the behavioral sessions at a dose of
0.1 mg/kg. Clozapine (Novartis, Switzerland), dissolved
in 1 N acetic acid (1.5 ml/10 mg) and diluted with saline,
was administered 30 min prior to the behavioral sessions
at a dose of 2.5 mg/kg. Imipramine (Sigma, Israel),
diluted with saline, was administered 30 min prior to the
behavioral sessions at a dose of 10 mg/kg. Olanzapine
(Eli Lilly Laboratories, USA), dissolved in 1 N tartaric
acid (15 mg/10 ml) and diluted with saline, was adminis-
terated 30 min prior to the behavioral sessions at a dose of
0.6 mg/kg. Paroxetine (Sigma, Israel), diluted with
distilled water, was administered 30 min prior to the
behavioral sessions at a dose of 7 mg/kg. No-drug controls
received an equivalent volume of the corresponding
vehicle.

Experimental design
Experiments 1, 2 and 3 tested the effects of haloperidol,
clozapine and imipramine, respectively, on LI. Each
experiment included four experimental groups in a 2 × 2
factorial design with main factors of pre-exposure (0, 40)
and drug (vehicle, [haloperidol, clozapine or imipra-
mine]). Experiment 4 tested the effects of vehicle,
haloperidol, clozapine and imipramine on FST. Experi-
ment 5 tested the effects of olanzapine and paroxetine on
LI. It included six experimental groups in a 2 × 3 factorial
design with main factors of pre-exposure (0, 40) and
drug (vehicle, olanzapine, paroxetine). Experiment 6
tested the effects of vehicle, paroxetine and olanzapine
on FST.

Statistical analysis
In LI experiments, times to complete licks 51–75 and
suppression ratios were analyzed with two-way ANOVAs,
with main factors of pre-exposure (0, 40), and drug (two
levels in Experiments 1–3 and three levels in Experiment
5), followed in cases of significant interactions by post-hoc
two-tailed t-tests, based on the error term derived from
the ANOVA, comparing the PE and the NPE groups
within each drug condition. In the FST, the duration of
immobility was analyzed with one-way ANOVAs, followed
by t-tests, based on the error term derived from the
ANOVA, assessing the difference between the vehicle
and treatment groups.

Results
Experiment 1: The effects of 0.1 mg/kg haloperidol on LI
The four experimental groups did not differ in their times
to complete licks 51–75 before tone onset (all P > 0.5;
mean A period = 7.8 s). Figure 1 presents the mean
suppression ratios of the vehicle- haloperidol-treated
PE and NPE groups. As can be seen, there was no
LI, in the vehicle-treated rats, whereas
haloperidol-treated rats exhibited LI, i.e. lower suppres-
sion of the PE as compared to the NPE group. This was
supported by a significant main effect of pre-exposure
[F(1,41) = 5.19, P < 0.05], and a significant pre-ex-
posure × drug interaction [F(1,41) = 4.23, P < 0.05], as
well as by post-hoc comparisons which revealed a
significant difference between the PE and the NPE
groups in the haloperidol t(41) = 2.84, P < 0.01 but not in
the vehicle t(41) = 0.16, NS, condition.

Experiment 2: The effects of 2.5 mg/kg clozapine on LI
The four experimental groups did not differ in their times
to complete licks 51–75 before tone onset (all P > 0.5;
mean A period = 5.2 s). Figure 2 presents the mean
suppression ratios of the vehicle- haloperidol-treated PE
and NPE groups. As can be seen, there was no LI in the
vehicle-treated rats, whereas clozapine-treated rats exhib-
ited LI. This was supported by significant main
effects of pre-exposure \[F(1,42) = 11.22, \ P < 0.01\] and drug \[F(1,42) = 5.81, \ P < 0.05\], and a significant pre-exposure × drug interaction \[F(1,42) = 4.24, \ P < 0.05\].

Post-hoc comparisons confirmed the existence of LI in the clozapine \(t(42) = 3.79, \ P < 0.01\) but not in the vehicle \(t(42) = 0.96, \) NS, condition.

Experiment 3: The effects of 10 mg/kg imipramine on LI
The four experimental groups did not differ in their time to complete licks 51–75 before tone onset (all \(P_s > 2\); mean A period = 8.5 s). Figure 3 presents the mean suppression ratios of the vehicle- or imipramine-treated PE and NPE groups. As can be seen, there was no LI in both conditions. ANOVA yielded no significant outcomes [pre-exposure, \(F(1,36) = 0.49\); drug, \(F(1,36) = 3.10\); pre-exposure × drug interaction, \(F(1,36) = 0.28\); all NS].

Experiment 4: The effects of 0.1 mg/kg haloperidol, 2.5 mg/kg clozapine and 10 mg/kg imipramine on FST
Figure 4 presents the mean duration of immobility in the FST in the vehicle, haloperidol-, clozapine- and imipramine-treated groups. As can be seen, haloperidol increased, whereas imipramine and clozapine decreased, the duration of immobility. This was supported by a significant main effect of drug \([F(3,28) = 19.87, \ P < 0.001]\), and subsequent post-hoc tests, which yielded a significant difference between the vehicle and haloperidol \(t(28) = 4.51, \ P < 0.001\); vehicle and clozapine \(t(28) = 2.11, \ P < 0.05\); and vehicle and imipramine \(t(28) = 2.24, \ P < 0.05\) groups.
Experiment 5: The effects of 0.6 mg/kg olanzapine and 7 mg/kg paroxetine on LI

The six experimental groups did not differ in their times to complete licks 51–75 before tone onset (all $P$s > 0.1; mean A period = 9.4 s). Figure 5 presents the mean suppression ratios of the vehicle-, olanzapine- or paroxetine-treated PE and NPE groups. As can be seen, there was no LI in the vehicle and paroxetine-treated rats, whereas olanzapine-treated rats exhibited LI. This was supported by a significant main effect of drug [$F(1,35) = 11.41$, $P < 0.001$] and a significant pre-exposure $\times$ drug interaction [$F(2,35) = 3.941$, $P < 0.05$]. Post-hoc comparisons confirmed the existence of LI in the olanzapine $t(35) = 3.34$, $P < 0.01$ but not in the vehicle $t(35) = 0.33$, NS, or the paroxetine $t(35) = 0.67$, NS, conditions.

Experiment 6: The effects of 7 mg/kg paroxetine and 0.6 mg/kg olanzapine on FST

Figure 6 presents the mean duration of immobility in the FST in the vehicle, paroxetine and olanzapine groups. As can be seen, both paroxetine and olanzapine decreased the duration of immobility. This was supported by a significant main effect of drug [$F(2,21) = 6.29$, $P < 0.01$] and subsequent post-hoc tests, which yielded a significant difference between the vehicle and paroxetine $t(21) = 2.95$, $P < 0.01$ and the vehicle and olanzapine $t(21) = 3.18$, $P < 0.01$ conditions.
Discussion

In agreement with previous reports, we found that imipramine, clozapine and paroxetine reduced, while haloperidol increased, immobility in the FST (Borsini et al., 1985; Gorka and Janus, 1985; Kawashima et al., 1986; Noda et al., 1997; Sanchez and Meier, 1997; Redrobe et al., 1998; Papp and Wieronska, 2000; Renard et al., 2001), and that clozapine and haloperidol potentiated LI under conditions that did not lead to LI in controls (e.g. Weiner et al., 1996, 1997; Trimble et al., 1998; Shadach et al., 1999, 2000), whereas imipramine had no such effect (Dunn et al., 1993). With regard to olanzapine, it has been shown that this drug reversed LI disruption induced by systemic amphetamine administration (Goselin et al., 1996) or entorhinal cortex lesion (Couvyreau et al., 2000). The present result provides the first demonstration that this drug has the capacity to potentiate LI under parametric conditions that do not yield LI in no-drug controls, as has been shown for all other APDs tested to date. Likewise, the result with paroxetine is the first demonstration that an SSRI does not potentiate LI when administered in both the pre-exposure and conditioning stages. It should be noted that the SSRIs fluoxetine and sertraline were reported to potentiate LI when administered only in the pre-exposure stage (Jakob and Rochford, 1995; Loskutova, 1998). This inconsistency could stem from the different parametric manipulations used to reduce LI in no-drug controls in these reports and the present study (low number of pre-exposures versus high number of conditioning trials), or because some action of the SSRIs in conditioning overrides their action in pre-exposure (we have recently shown such a competition between the pre-exposure-based and the conditioning-based actions of atypical APDs, Weiner et al., 2003). In any event, even if SSRIs potentiate LI via effects in pre-exposure, such a potentiating action is different from that produced by APDs, which produce LI potentiation via effects in the conditioning stage (e.g. Peters and Joseph, 1993; Weiner et al., 1997; Shadach et al., 1999, 2000); when administered in pre-exposure alone, typical APDs have no effect, and atypical APDs disrupt LI (Weiner and Feldon, 1987; Weiner et al., 1987, 1997, 2003; Shadach et al., 1999, 2000).

While the effects of each of the classes of drugs in each of the two standard tests separately have been shown previously, the aim of the present experiments was to show that the pattern of their effects in both tests would reveal a distinct behavioral ‘fingerprint’ that could discriminate between the three classes of drugs. This was indeed the case: haloperidol increased immobility in the FST and potentiated LI, clozapine and olanzapine reduced immobility in the FST and potentiated LI, and imipramine and paroxetine reduced immobility in the FST and failed to potentiate LI.

Clearly, these results are preliminary, and further studies with additional compounds and doses from each of the three classes of drugs are needed to confirm the distinct patterns of action of typical APDs, atypical APDs and antidepressants in LI and FST. In particular, while the capacity of APDs to potentiate LI, and the capacity of antidepressant drugs to reduce immobility in FST are well established, data on the effects of APDs on FST, as well as on the effects of antidepressants on LI, are relatively sparse. Likewise, it would be of interest to identify what common mechanism(s) underpin the behavioral effects in these two different paradigms. It is of interest to note in this context that we found that lesions that potentiate LI under conditions used here (nucleus accumbens core, basolateral amygdala) also increased immobility in the FST (unpublished results).

If confirmed with additional drugs, the ‘LI–FST assay’ would have several advantages in comparison to the existing behavioral assays which have been shown to dissociate between typical and atypical APDs (Bakshi and Geyer, 1995; Noda et al., 1995; Swerdlow et al., 1996; Sams-Dodd, 1997): First, while the existing assays are sensitive to atypical but not to typical APDs, the combined use of LI and FST would discriminate between typical and atypical APDs without losing selectivity for APDs in general. Second, LI and FST would not rely on pharmacological means to elicit the behavioral index of both the common (antipsychotic) and the discriminating (antidepressant) activity. In addition, the potential capacity of LI and FST to discriminate between atypical APDs and antidepressants would be advantageous in view of the clinical findings that atypical APDs exert an antidepressant action (e.g. Hillert et al., 1992; Tollefson et al., 1998; Tollefson and Sanger, 1999), but antidepressants are not effective, and may be deleterious, in treating schizophrenia (Siris et al., 1978; Plasky, 1991). Such a discrimination would enable a better screening of atypical APDs, and possibly shed light on the usefulness of antidepressant treatment in schizophrenia. Finally, the identification of the mechanisms of action of APDs and antidepressants in the two behavioral paradigms may shed light on the relationship between schizophrenia symptoms and depression in general (Nelson and Bowers, 1978; Siris et al., 1988, 1991; Plasky, 1991).

It should be pointed out that Noda et al. (1995) used the FST to dissociate between typical and atypical APDs, but such a dissociation was deemed to require PCP administration; moreover, attenuation of PCP-induced increase in immobility was also obtained with some antidepressants (Noda et al., 1997). Thus, PCP administration does not seem to confer an advantage to the FST in comparison to a non-pharmacological FST, because both can dissociate between typical and atypical APDs, but not between the latter and antidepressants. Our results
suggest that the addition of a non-pharmacological test that is specific and selective for APDs, i.e. LI, may solve the confounding inherent in the FST, and allow the differentiation between the three classes of drugs.

In conclusion, we suggest that behavioral screening of drugs that share some clinical actions but differ in others, as is the case with typical and atypical APDs or atypical APDs and antidepressants (as well as with many other classes of drugs, e.g. antidepressants with or without anti-obessional activity), may be considerably improved by using tests that provide distinct behavioral measures of the shared and dissimilar actions that form specific patterns of behavioral drug effects. The present findings provide a preliminary evidence that the ‘LI–FST assay’ may fulfill the criteria of such a ‘pattern-oriented’ screening strategy and thus provide a useful tool for screening novel agents with an atypical antipsychotic profile distinct from that of typical APDs and antidepressants. While such a ‘pattern-oriented’ screening strategy was achieved here by using different behavioral tests, it can also be achieved by using different behavioral measures derived from a single test (see Shadach et al., 2000; Weiner et al., 2003).

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References


