Novel oral drug administration in an animal model of neuroleptic therapy

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Abstract

A novel method of oral drug administration was used in a neuroleptic animal study. Seventy male Sprague-Dawley rats were randomly subdivided into four groups, which were treated with clozapine, haloperidol, diazepam or a vehicle solution (5% sucrose solution). Oral drug treatment was achieved by training the rats to drink the drug of choice mixed with five percent sucrose or vehicle solution from a syringe. Within 3–4 weeks the haloperidol group developed vacuous chewing movement, which did not disappear with discontinuation of the drug. Significant weight gain was observed for all drug groups in relation to the control group, whereas only the diazepam group showed a significant increase in response latency on the disengage test of sensorimotor function, which disappeared with drug withdrawal. A novel means of testing the motivational status showed that all drug-treated groups engaged in eating chocolate before grooming (t = 11.69, p < 0.001), whereas the control group showed no specific tendency towards either task. Furthermore, there was a significant delay in grooming for the haloperidol group compared to the other drug groups and controls. In conclusion, a novel method of oral drug administration with minimum stress was introduced that was sufficient to cause the described changes in behavioural parameters. Additionally, the combination of tests used provided an efficient discrimination between the behavioural effects of clozapine, haloperidol and diazepam in rodents.

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1. Introduction

Previous studies have successfully used oral drug administration in neuroleptic models, where the drug of choice was either administered by gavage or added to the drinking water, after a deprivation period to ensure compliance (Gao et al., 1997; Ossowska et al., 2002; Schmitt et al., 1999; See and Chapman, 1994; Sun and Lau, 2000). The following study introduces a novel alternative to the previously used method, where a mixture of 5% sucrose solution and neuroleptic drug (clozapine, haloperidol or diazepam) was given to the test animals using a syringe for oral administration. This new method permits accurate drug administration with minimum stress to the animals.

Typical and atypical neuroleptic drug administration in rodents is a commonly used animal model to mimic some of the clinical features seen with neuroleptic drug administration. Previous rodent studies have shown that treatment with typical (e.g. haloperidol), but not with atypical neuroleptics (e.g. clozapine), will induce extrapyramidal behavioural effects such as vacuous chewing movements (Casey, 2000; Gao et al., 1997; Turrone et al., 2002). This phenomenon is considered to be an analogue to clinical tardive dyskinesia, which can occur with long term treatment of typical antipsychotics (Casey, 2000; Turrone et al., 2002). Previous rodent studies have shown that clozapine has a similar anxiolytic effect to benzodiazepines in rodents, despite its clinical use as a neuroleptic (Sun and Lau, 2000; Weiner et al., 2003). Furthermore, it generally does not cause extrapyramidal side effects, which are not uncommonly seen after typical neuroleptics (Turrone et al., 2002). Whilst cloza-
pine has a similar anxiolytic action to benzodiazepines it potentiates latent inhibition (Weiner et al., 2003), unlike benzodiazepines. Latent inhibition is defined as a retarded conditioning to a stimulus as a consequence of its inconsequential pre-exposure (Russig et al., 2003; Weiner et al., 2003; Zuckerman et al., 2003). While the use of benzodiazepines is not a standard clinical procedure, clinical trials have shown that benzodiazepines may be beneficial alone for the acute treatment of positive symptoms or in combination with neuroleptics in the treatment of schizophrenia (Wassef et al., 1999, 2000; Wolkowitz and Pickar, 1991). This study therefore compared the behavioural patterns of atypical (e.g. clozapine) and typical neuroleptics (e.g. haloperidol) and benzodiazepines (e.g. diazepam).

Previous studies have concentrated on behavioural parameters, such as latent inhibition, forced swimming and weight gain, which have been used to dissociate between drug-specific side effects (Russig et al., 2003; Schmitt et al., 1999; Weiner et al., 2003; Zuckerman et al., 2003), but the motivational status in rats administered these drugs has not been tested previously. A novel assessment of motivational status was created to assess the effect of drug treatment on exploratory as well as motivational behaviour.

The purpose of this study was to use a novel means of oral drug administration in rodents to investigate behavioural effects (including possible extrapyramidal features) associated with clozapine, haloperidol and diazepam. A time period of 7 weeks was chosen to ensure the effectiveness of the drug therapy and to test for the reversibility of behavioural effects by withdrawing drug treatment. The effectiveness and specificity of drug treatment was tested by comparing several behavioural parameters (including weight, response latency and motivational status) between drug- and control-treated groups.

2. Material and methods

2.1. Animals

Seventy male Sprague-Dawley rats (LAB-animal services, Perth, Australia), weighing approximately 350 g on arrival were used in these experiments. Rats were housed in groups of five in an animal house under constant room temperature and humidity on a 12 h light–dark cycle (light on 06:00–18:00), and had access to food and water ad libitum. All rats were given a habituation time of 2 weeks before being tested.

2.2. Drugs

Clozaril 25 tablets (clozapine 25 mg, Novatis Pharmaceuticals Australia) were totally pulverised and completely dissolved by heating the solution (37°C) whilst constantly stirring. The non-active ingredients in the clozapine were thought to be negligible and were not accounted for in this study. Other drugs used were haloperidol (Sigma-Aldrich, Australia) and diazepam (10 mg/2 ml, David Bull Laboratories, UK). All drugs were dissolved and mixed with 5% sucrose solution to disguise the taste of the drugs. The vehicle solution for the control group therefore consisted of 5% sucrose solution.

2.3. Drug administration

Oral drug administration was used in this study to minimise the stress and pain levels for the animals, since drugs were given daily over a time period of 7 weeks. Each rat was trained to drink 2 ml of 5% sucrose solution from a disposable syringe on a daily basis for 1 week or until each rat had taken the 2 ml of fluid from the syringe on three consecutive days (see Fig. 1). The animals very easily adapted to this administration procedure. Due to the individual drug administration it was possible to monitor closely the daily drug intake. The total amount of time for an individual rat to drink the drug/vehicle solution was 22 s to 1.30 min, since the chosen volume was small enough to be taken at once and within a brief time period.

When all rats were trained in the administration procedure, cages were randomly subdivided into control (vehicle solution), clozapine (20 mg/kg), haloperidol (2 mg/kg) and diazepam (0.5 mg/kg) groups. The selected drug concentrations were based on previous studies (Gao et al., 1997; Schmitt et al., 1999).

At 4 weeks, five rats from each drug and control group were sacrificed and five rats from each of the drug groups were taken off each drug and fed vehicle solution alone for the remaining 3 weeks, as were the remaining control rats (see Table 1). After all behavioural tests were completed at 7 weeks, all remaining animals were sacrificed for post-mortem analysis.

2.4. Behavioural procedure

2.4.1. Animal monitoring

Rats were monitored daily and weighed twice a week from the time of arrival until the end of the project (as recom-
### Table 1: Drug groups and exposure time

<table>
<thead>
<tr>
<th>Number of animals</th>
<th>Drug group</th>
<th>Daily dose (mg/kg)</th>
<th>Exposure time to drug (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Control</td>
<td>Vehicle solution</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>Clozapine</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>Clozapine</td>
<td>20</td>
<td>7</td>
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<tr>
<td>10</td>
<td>Haloperidol</td>
<td>2</td>
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<td>10</td>
<td>Haloperidol</td>
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<td>7</td>
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<tr>
<td>10</td>
<td>Diazepam</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>Diazepam</td>
<td>0.5</td>
<td>7</td>
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</tbody>
</table>

(mended by the Institutional Ethical Committee). This was indispensable, since the animals displayed a fast growth velocity and appropriate daily drug concentration was calculated from their weight. Each cage contained similar environmental enrichments with plastic tubes, wooden blocks, sunflower seeds and toilet rolls. Additionally all animals were checked for signs of over-grooming, which can indicate increased stress levels or boredom, and for any adverse effects from the drug treatment.

2.5. Sensory-motor disengage test

The sensory-motor disengage test involves the rat engaging in an activity whilst applying a sensory stimulus to the whiskers on each side. This test is done to measure the response to a stimulus, whilst sensory-motor gating takes place.

Baseline testing was done 2 weeks after arrival and before drug or vehicle administration was commenced. Following administration of the solutions, all animals were tested at 2, 4, 5 and 7 weeks.

2.6. Chocolate disengage test

The test was performed in a testing cage that was identical to the home cage but had a 2.5 g piece of plain chocolate placed on the bedding. Each rat was placed in the cage individually and given time to explore the cage. Once the rat began to eat the chocolate the whisker was touched with a probe containing a cotton bud tip in such a fashion so that the rat could not see or hear the stimulus before it was applied and the ensuing response time was measured. This was done for each side three times and the average was taken.

2.7. Grooming disengage test

As described above this test was executed in the same testing cage but this time the sensory motor response was measured whilst the animal was grooming.

2.8. Motivational status

The animal was placed into the testing cage and the time was measured until the rat either engaged into grooming or eating chocolate, as well as to which event occurred first. This was used as a measurement of the anxiety levels of the tested animal as well as of their motivational behaviour.

2.9. Statistical analysis

The weight measurements and results from the behavioural tests were analysed using two-way repeated measures ANOVA (Stat View 5.0.1) and post-hoc Fischer’s t-tests. The withdrawal period data were analysed in the same manner excepting that data was blocked (block 1 comprising weeks 0-4 and block 2 comprising weeks 5-7). All data are shown as means with standard error of mean. For the comparison of latency of onset in motivational status relative to grooming onset, a paired t-test was performed for each group. For all analyses p < 0.05 was accepted as significant.

3. Results

3.1. Overall behaviour

After habituation, all rats cooperated with the daily drug administration without any difficulties. This became obvious when the tests animals lined up at the rim of the cage as soon as the cage was opened to receive their daily solution. The total time to administer 2 ml of solution to 70 rats was decreased from 3 h initially to 45 min by week three due to the above-mentioned habituation to this procedure.

Overall, all participating animals displayed minimal levels of stress, which was reflected by the successful voluntary participation in the daily drug administration and the bi-weekly behavioural testing.

3.2. Vacuous chewing movement

Vacuous chewing movement is defined as purposeless mouth openings in the vertical plane, with or without tongue protrusion (Turrone et al., 2002). This feature is thought to be an analogue to tardive dyskinesia, a side effect experienced with haloperidol, since it produces some but not all of the critical features of this clinical syndrome (Casey, 2000). The onset of vacuous chewing movement occurs within the first 2-4 weeks of drug treatment. In our haloperidol group it was noted that the rats had developed vacuous chewing movement within 3-4 weeks of drug treatment (at 4 weeks, F(3,35) = 92.9, p < 0.001), when compared to control and the other drug groups. This phenomenon did not disappear with discontinuation of the drug.

3.3. Weight gain

The mean weight gain after 4 weeks of treatment was only significantly increased for diazepam (460 ± 9.8 g) (F(3,206) = 4.09, p = 0.0075), when compared to control (417 ± 15.5 g), clozapine (415 ± 10 g) and haloperidol (443 ± 9.4 g) treated groups. But after 7 weeks of treat-
ment all drug groups (clozapine = 567 ± 16.7 g, haloperi-
dol = 594 ± 9.8 g and diazepam = 578 ± 11.4 g) displayed
a significant, but similar weight increase ($F_{3,106} = 9.07, p < 0.001$; all post-hoc tests $p < 0.001$) in contrast to the
ccontrol group (507 ± 24.3 g). At 7 weeks the withdrawal
groups for each drug treatment were not significantly differ-
et in weight when compared to the drug treatment groups
($F_{1,13} = 2.77, p = 0.12$).

3.4. Disengage tests

For disengage tests, involving chocolate administration
and grooming, only the diazepam group showed a signifi-
cant bilateral delay in response latency when compared to
the other treatment groups ($F_{1,13} = 19.9, p < 0.001$; post-hoc
$p < 0.05$). This response delay was totally reversed after drug
withdrawal at week 5 ($F_{1,13} = 3.88, p < 0.001$) as is displayed
in Fig. 2(A).

3.5. Motivational status

All drug treatment groups engaged in the chocolate task
significantly quicker in comparison to the control group
($F_{3,163} = 17.3, p < 0.001$) (see Fig. 2(B)). For the grooming
onset there was a significant increase in response time for the
haloperidol group in comparison to the diazepam and cloza-
pine treated animals ($F_{3,66} = 4.307, p = 0.0078$, both post-
hoc comparisons $p < 0.0001$). This is illustrated in Fig. 2(C).
Paired $t$-tests showed that all drug treatment groups en-
gaged in the chocolate task before they groomed (clozapine:
$t = 5.297, p < 0.001$; haloperidol: $t = 5.893, p < 0.001$; di-
zepam: $t = 7.912, p < 0.001$), whereas the control group
showed no specific tendency towards either task.

4. Discussion

4.1. Drug administration

The behavioural results indicate that the route of drug ad-
nistration was effective, with the amount of drug delivered
being high enough to induce drug-related side effects in the
test animals. The daily contact with the rats influenced the be-
havioural testing in a positive manner, since the animals were
used to being handled on a daily basis without being harmed
in any way. This was reflected by the voluntary participation
in both drug administration and behavioural testing.

This study aimed to simulate a clinical setting by via the
selected doses and oral route of administration (Turrone et al.,
2002). Furthermore it was felt that the drug administration
had to be commenced for a minimum of 2–4 weeks, since
the onset-delay for extrapyramidal side-effects, as seen with
the use of typical neuroleptics, is between 2 and 4 weeks
(Ossowska et al., 2002; Schmitt et al., 1999; Turrone et al.,

![Fig. 2. (A) Chocolate disengage test: drug withdrawal for the diazepam group at week 5. When a subgroup of the diazepam treated rats was taken off the drug,
within a week the response delay was reversed ($p < 0.001$). (B) Chocolate task. All drug groups engaged in the chocolate task significantly quicker than the
control group ($p < 0.001$), which showed no preference to commence either grooming or eating chocolate. (C) Grooming task. The haloperidol group showed
a significant delay in grooming onset compared to the clozapine and diazepam groups ($p = 0.0078$).]
2002; Weiner et al., 2003). Additionally, it has been shown that acute drug administration via intravenous or intraperitoneal injection causes acute tolerance, which is not seen with oral treatment (Sun and Lau, 2000).

Previous studies have shown that daily intraperitoneal drug injection has a negative influence on the assessment of behavioural testing due to severe stress effects (Schmitt et al., 1999; Stahle and Ungerstedt, 1986). Therefore oral drug administration in drinking water has become the favoured technique for neuroleptic drug administration, where assessment of behavioural parameters play a role in study outcome. Since in most cases rats are housed in groups of 3–5 it is hard to control the individual daily intake of each rat from a drinking bottle. Serum screening has been used to monitor the average intake of drug on a weekly basis, but due to the extraction of venous blood the animals are exposed to the same type of stressor as with the intraperitoneal injection technique. This study proposed a new model of oral drug administration that is based on the drinking water technique, but allows accurate individualised drug administration. To mimic oral intake in a clinical setting, the drug doses were given once a day in 2 ml of vehicle solution, which the rats consumed at once in a time period of 22 s to 1.30 min per administration. Serum levels were not measured since the applied doses had been successfully used in previous studies (Gao et al., 1997; Schmitt et al., 1999) and the presence of extrapyramidal and anxiolytic effects in the treated animals indicated that the dose employed had been sufficient.

4.2. Weight gain

The significant increase in weight gain for all drug groups versus controls showed that the treatment with clozapine, haloperidol and diazepam had no negative impact on the state and development of the rats (Schmitt et al., 1999; Stahle and Ungerstedt, 1986). So far, increased weight gain has only been described as a side effect for clozapine (Birt, 2003; Corbett et al., 1993; Schmitt et al., 1999), but not for haloperidol (Schmitt et al., 1999) or diazepam (D’Mello et al., 1999). Whilst the increased weight gain for clozapine and haloperidol might be associated with their effect on serotonin levels in the brain (Busatto and Kerwin, 1997), the effect of diazepam on weight is not clear, especially since after one month of drug treatment there was only a significant increase in weight in the diazepam group, relative to the other drug-treated and control groups. This may be due to the increased inactivity caused by the sedentary effect of diazepam.

4.3. Sensory-motor test

An increased onset latency to respond to an external stimulus was only observed for diazepam, and not for any of the other treated groups. This may suggest that whilst diazepam and clozapine have an anxiolytic effect in rodents (Sun and Lau, 2000), only diazepam causes enough sedation to cause a significant response delay. This observed effect was reversible within 2 weeks, when the drug was discontinued and is consistent with clinical studies (Follesa et al., 2002).

4.4. Motivational task

The motivational status test measured the time delay until the test animal started to groom or to eat the chocolate when placed into the testing cage. This test for the appetitive behaviour, which is associated with the decrease in latency to engage in eating the chocolate, which was increased in all drug groups, as well as the exploratory behaviour, when placed into a new environment.

4.5. Chocolate task

There was a significant decrease in onset in engaging in the consumption of chocolate for all drug groups, when compared to the control group. This phenomenon was hypothesised to be either due to an increase in appetite due to the medication, which is a common side effect with clozapine (Birt, 2003) and haloperidol, and/or a decrease in inhibition (Sachs, 1988). The latter may be due to the anxiolytic effect of these drugs, as has been previously described for clozapine and diazepam, but not for haloperidol (File, 1988; Gardener and Guy, 1984; Sachs, 1988). This increase in appetite may explain the observed increase in weight for all drug groups compared to the control groups, since all other conditions were the same for all tested animals.

In addition, previous studies (Schmitt et al., 1999; Sun and Lau, 2000; Willner, 1997; Yadid et al., 2000) have shown that environmental stressors, such as drug administration, can cause a gradual decrease in consumption of sucrose solution and decrease in response to reward. In this study all test animals showed no decrease in appetitive behaviour, which was seen as a positive indicator of the well being of the animals.

4.6. Grooming behaviour

Grooming has been previously described as either an indicator for social interactions or a marker of feeling safe in an environment (File, 1988; Li et al., 2003). In this study a significant increase in onset-delay for the haloperidol group was found when compared to the clozapine and diazepam treated rats. This finding complements the observations of previous studies, which showed that drugs, such as atypical neuroleptics and benzo diazepines, have an anxiolytic effect in rodents and therefore increase social and explorative behaviours by reducing anxiety associated with an aversive environment (File, 1988; Gardener and Guy, 1984; Sammis-Dodd, 1995). In addition it has been shown (Corbett et al., 1993) that clozapine increases social behaviour in rodents, whereas haloperidol seems to decrease it, which was observed with the increased onset delay in the haloperidol group.

In conclusion, the main purpose of this study was to introduce a novel oral drug administration technique. Further-
more, a series of behavioural tests were used to show the efficiency of the applied drug administration as well as emphasise the dissociation of different behavioural profiles of clonazepam, haloperidol and diazepam in rodents. In summary, diazepam showed a significant response delay in the sensory motor test and a decreased time delay in the chocolate and grooming onsets, clonazepam showed no delay in the sensory motor test and displayed a decreased time delay in the chocolate and grooming and, haloperidol showed no delay in the sensory motor test and showed decreased time delay in the chocolate, but not a decrease in time delay for grooming onset. In addition, the haloperidol group developed vacuous chewing movements within 4 weeks of drug administration.

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