The role of hypothalamic pathways in the metabolic side effects of Olanzapine

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Chapter 2.2

Technical issues concerning peripheral and central administration of Olanzapine in rats

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*These authors contributed equally to this work.

In preparation.
ABSTRACT

Determining the optimal mode of administration of a drug is an important aspect in developing an animal model. The chemical and pharmacokinetic properties of a drug can markedly influence this. In this article, we describe some of the technical issues encountered with different modes of administration of Olanzapine (Ola) whilst developing rat models for the metabolic side effects of Ola.

Due to the short half-life of Ola in rats, simple daily injections do not result in chronic drug exposure that is comparable to humans. Previous attempts to circumvent this issue by using implantable osmotic minipumps, proved suboptimal due to degradation of Ola in an acidified solution kept at body temperature. In vitro experiments revealed that the stability of Ola was considerably higher at room temperature compared to body temperature. Furthermore, central administration of Ola proved challenging: after intracerebroventricular (ICV) administration to male Wistar rats, significant leakage of Ola into the general circulation and poor penetration of Ola into brain tissue was observed.

The aim of this paper is to contribute to the development of future rat models by raising awareness of the technical issues that may arise with drug administration to rats in general and of Ola specifically. Different options for peripheral and central administration of Ola in rats are discussed, together with their advantages and disadvantages.
INTRODUCTION

Since its introduction to the market in 1996, Olanzapine (Ola) has become a widely prescribed drug in the treatment of schizophrenia and bipolar disorder (Hermann et al. 2002; Leucht et al. 2009; Scherk et al. 2007; Smith et al. 2007). Unfortunately, similar to other atypical antipsychotic drugs (AAPDs), the treatment benefits of Ola were soon overshadowed by metabolic side effects: significant weight gain, dyslipidemia and hyperglycemia frequently occur and have a negative effect on patients compliance (Leucht et al. 2009; Lieberman et al. 2005; Newcomer 2007; Parsons et al. 2009; Rummel-Kluge et al. 2010). Moreover, this increases their cardiovascular risk (Hennekens et al. 2005; Newcomer 2007). Because the therapeutic effect of Ola remains superior to other AAPDs that show less weight gain liability (Komossa et al. 2010; Leucht et al. 2009), research into the mechanisms underlying Ola-induced weight gain is of great importance.

Compared to clinical studies, animal models offer additional opportunities to elucidate the mechanisms underlying the metabolic side effects of Ola, which has led to development of rat models by several research groups (for review see Boyda et al. 2010). In the process of developing suitable rat models for the metabolic side effects of Ola, we encountered several technical issues with different modes of administration of Ola, including administration directly into the brain, which were mostly due to the chemical and pharmacokinetic properties of the drug. This paper describes the issues encountered, in order to serve as a cautionary note for other researchers that are interested in investigating the effects of Ola in rats. In addition, a number of different approaches to circumvent these issues are described, together with the advantages and disadvantages of each approach.

Part 1: Chronic peripheral administration

One of the difficulties in accurately modeling Ola-induced weight gain in rats is the marked difference in half-life of the drug, which is ~ 33 hours in humans, compared to ~ 2.5 hours in rats (Aravagiri et al. 1999; Callaghan et al. 1999). Once or twice daily administration of Ola in rats, therefore, results in marked fluctuations in plasma levels throughout the day and does not resemble drug exposure in the human situation. Furthermore, effects occurring at peak plasma levels (~30 min) may disappear rapidly after the drug is cleared from the blood.

In order to circumvent this issue and achieve stable plasma levels for a longer period of time, we first used subcutaneously implanted osmotic minipumps to continuously infuse an Ola solution and model Ola-induced weight gain in rats (van der Zwaal et al. 2008). However, the contents of the osmotic minipumps used to administer Ola for 4 weeks had clearly changed
color over the course of the experiment, and we observed a similar discoloration when Ola solution was stored in an incubator at 37°C. This change in color was most likely due to the degradation of the drug, as it is known to be sensitive to oxidation (Olesen and Linnet 1998). Although it was previously shown that an acidified solution of Ola in water (~ 0.1 mg/ml) kept at room temperature for 4 days showed no signs of degradation (Terry et al. 2008), the Ola solution used in the minipump experiments had a much higher concentration (up to 42 mg/ml), and was exposed to a higher temperature for a longer period of time. To estimate the speed at which degradation of Ola took place in the interior of an implanted osmotic minipump and to determine the influence of temperature on this process, the following in vitro experiment was performed.

MATERIALS AND METHODS (part 1)

A stock solution was prepared by solubilizing Ola (Chempacific Corp, Baltimore, USA) in a minimum quantity of hydrochloric acid and diluting it with purified water to a concentration of 42 mg/ml, after which pH was set at ~ 5.5 with 1M NaOH. The concentration of this solution was identical to that of the solution in the minipumps implanted in rats receiving the highest dose in the minipump paradigm (van der Zwaal et al. 2008). Next, the solution was divided over 9 eppendorf containers. One container was immediately frozen and stored at -20°C. Half of the remaining containers was then stored at 37°C in an incubator, and the other half at room temperature (18°C). Every week thereafter, two containers were frozen and stored at -20°C: one previously stored at room temperature and the other previously stored in the incubator.

After 4 weeks, all containers had been frozen and the Ola concentration in each container was determined by performing HPLC analysis followed by ultraviolet (UV)-detection, as described previously (van der Zwaal et al. 2008). Briefly, a calibration curve (0-200 ng/ml) was prepared, a control solution of Ola (40 ng/ml, SKML, the Netherlands) was used as quality control and 500 ng promazine HCl (Sigma, Australia) was added to all samples as internal standard. After liquid-liquid extraction, plasma samples were analyzed by an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA). Lower detection limit was 10 ng/ml. Analysis was performed in duplo for each sample and in a single experimental run.

RESULTS (part 1)

Within one week, the color of the solutions kept in the incubator had already changed from
orange to green, whereas the solution kept at room temperature did not show any signs of discoloration until the end of week 4. This difference was clearly reflected in the values obtained with HPLC analysis (Figure 1). The concentration of Ola in the solution kept at room temperature showed only a slight decline (~10%) over the 4 weeks of the experiment, but at 37°C the concentration of Ola had declined by nearly 75%.

![Image of Figure 1]

**Figure 1:** Olanzapine concentrations of samples stored at either room temperature (18°C) or in an incubator (37°C). Data expressed as percentage of the concentration in the fresh sample (Mean ± S.E.M.).

**DISCUSSION (part 1)**

The results of this experiment offer an illustration of the extent to which degradation most likely occurs within the interior of an osmotic minipump containing an acidified solution of Ola in water. In addition, it explained the marked reduction in plasma levels of Ola that were observed after long-term (4 weeks) minipump infusion (van der Zwaal et al. 2008).

The influence of temperature on the degradation process appears marked, as degradation occurred much faster in the samples stored at body temperature than at room temperature. This implies that it is possible to chronically deliver a similar Ola solution using a different mode of administration, provided the solution is kept at room temperature in order to minimize degradation (for example, infusion using an external pump attached to an intragastric cannula via a swivel system attached to the home cage).
As rats will drink water throughout the dark phase, administration of an acidified Ola solution in drinking water results in drug exposure that is more comparable to the human situation than after daily injections, although not as constant as with continuous infusion (Perez-Costas et al. 2008). Degradation of Ola is unlikely to interfere with drug delivery in this paradigm, as water bottles are kept at room temperature and can easily be refreshed once a week before significant degradation has taken place. A disadvantage of this method, however, is the reduction in water intake that occurs secondary to the bitter taste of Ola (van der Zwaal et al. 2010).

Mixing Ola with powdered food available in the home cage of rats is a second option that has been used previously in chronic experiments (Victoriano et al. 2009). Although degradation is not an issue using this approach, because Ola is in a solid state, effects on food intake may occur secondary to altered flavour of the food, which could interfere with its effects on feeding. These flavour effects could be overcome by masking the bitter taste of Ola using, for example, saccharin mixed with the food. However, added sweetness itself could, in turn, have secondary effects on feeding. Such effects would not be expected when Ola (or a control solution) is mixed with cookie dough for example, which is offered several times a day in addition to regular chow (Weston-Green et al. 2011). In essence, this approach is equivalent to delivery via repeated daily injections, which is another option for chronic administration. However, due to the very short half-life, Ola would have to be administered several times a day in order to achieve fairly stable plasma levels.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Advantages</th>
<th>Disadvantages</th>
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</thead>
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<tr>
<td>Osmotic mini-pumps administering acidified solution of Ola in water</td>
<td>Simple procedure&lt;br&gt;Continuous infusion</td>
<td>Rapid degradation of Ola-solution at body temperature</td>
</tr>
<tr>
<td>Microinfusion pumps administering PEG400 solution of Ola</td>
<td>Continuous infusion&lt;br&gt;No degradation</td>
<td>Refilling of pump necessary every week due to limited reservoir volume</td>
</tr>
<tr>
<td>Infusion of acidified solution of Ola via Ig cannula using external pump</td>
<td>Solution stable&lt;br&gt;Continuous infusion</td>
<td>Labor-intensive</td>
</tr>
<tr>
<td>Ola mixed with drinking water</td>
<td>Solution relatively stable&lt;br&gt;Solution can be easily refreshed</td>
<td>Flavour effects affect water intake&lt;br&gt;Lower plasma levels of Ola in the light phase</td>
</tr>
<tr>
<td>Ola mixed with food available in home cage</td>
<td>No degradation</td>
<td>Flavour effects can affect food intake&lt;br&gt;Lower plasma levels of Ola in the light phase</td>
</tr>
<tr>
<td>Repeated administration of Ola in portion of palatable food</td>
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<td>Repeated injections of Ola (IP/SC)</td>
<td>Simple procedure</td>
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<tr>
<td>Depot injection of Ola-pamoate</td>
<td>Simple procedure</td>
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</tr>
<tr>
<td></td>
<td>Stable plasma levels</td>
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</tr>
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</table>

*Table 1: Advantages and disadvantages of different modes of chronic peripheral administration of Ola.*
A depot injection of Ola-pamoate that gradually releases Ola over time could be used as a convenient alternative, although this is not yet commercially available (Citrome 2009). In a recent paper, Shobo et al. used microinfusion pumps to administer a solution of Ola in polyethylene glycol 400 (PEG 400). They examined the stability of this solution (4 mg/ml) in an incubator at 37°C and showed it was stable for at least 42 days (Shobo et al. 2011). Therefore, using PEG 400 to solubilize Ola seems an attractive alternative to the acidified solution of Ola in water, although, due to the limited reservoir volume, pumps need to be refilled percutaneously every 7 days (Shobo et al. 2011).

Taken together, although rapid degradation of Ola in an acidified aqueous solution limits the use of osmotic minipumps for chronic administration, several options remain that can circumvent the issue of the short half-life of Ola in rats. These are summarized in table 1.

**Part 2: Central administration of Ola**

Intracerebroventricular (ICV) administration into the lateral ventricle of the brain is a frequently used method to differentiate between the peripheral and central effects of substances known to pass the blood-brain barrier. After Ola is administered peripherally, it is rapidly absorbed into the brain and maximum concentrations in brain tissue are higher than in plasma (approximately 5 times) (Aravagiri et al. 1999). In addition, the half-life in brain tissue is longer (approximately 5 hours) than it is in plasma (approximately 2.5 hours) (Aravagiri et al. 1999). Because of these pharmacokinetic properties and the fact that the brain plays an important role in the regulation of energy balance (Schwartz et al. 2000), we hypothesized that the metabolic effects that are observed after peripheral administration of Ola are mainly due to actions of the drug in the brain. To test this hypothesis, we investigated the effects of administration of Ola through an ICV cannula placed in the lateral ventricle of the brain.

To directly compare the effects of central administration to the effects previously observed after peripheral administration (van der Zwaal et al. 2012) an experiment was performed in which animals received Ola via intraperitoneal (IP) injections as well as injections via an ICV cannula (Experiment 1). Because, in a preliminary experiment, Ola failed to affect locomotor activity at ICV doses of 10 and 30 μg, a dose of 60 μg was administered in this experiment. For a rat of 300 g, this dose was approximately 20% of the peripherally administered dose of 1 mg/kg (which had previously been shown to affect locomotor activity and food intake after acute administration (van der Zwaal et al. 2012)). At the end of Experiment 1, plasma samples were collected and Ola concentration was determined in order to investigate
whether there was any leakage of Ola into the circulation after ICV administration of this dose.

In a study described previously, the effect of Ola on glucose metabolism after peripheral (intragastric, IG) administration was compared to that after ICV administration (Girault et al. 2012). In this study, IG infusion of Ola led to hyperglycaemia and insulin resistance, whereas none of these changes were observed after ICV administration (Figure 2).

![Graph showing glucose levels over time](image)

**Figure 2: Effects of Ola on glucose levels after IG and ICV administration (adapted from Girault et al. 2012).** Open circles=ICV-vehicle animals; black circles=ICV-Ola animals; open triangles=IG-vehicle animals; black triangles=IG-Ola animals. *p<0.05 for IG-Ola-treated animals vs vehicle-treated animals (ANOVA repeated measures).

Plasma levels of Ola were also measured at the end of this study (for details see (Girault et al. 2012)) and Ola appeared to be detectable in the plasma of over half of the animals receiving an ICV injection in this experiment (Table 2), indicating leakage into the circulation after ICV administration. After completion of this study, we decided to perform an additional experiment to investigate whether brain concentrations after ICV administration of Ola were comparable to those after IG administration (Experiment 2). As the brains of the ICV-treated animals in the original study had been used for staining, a different set of animals was used to examine this.
Experiment 1
A week after arrival, nine naive male Wistar rats (275-300 g) were equipped with an intra-abdominal transmitter (TA10TA-F40, Data Science International, St. Paul, Minnesota, USA) and an ICV cannula aimed at the lateral ventricle (C313G, Plastics One, Roanoke, USA). Surgery was performed under fentanyl/ fluanisone (Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium, 0.1ml/100g intramuscular) and midazolam (Dormicum®, Roche, Woerden, The Netherlands, 0.05 ml/100g IP) anesthesia. Animals were then individually housed (54 x 26 x 35 cm plexiglas cages with wood shaving as bedding) in a specific pathogen free facility under a 12 h/12h light/dark cycle (lights on at 03:00) at 21 ± 2°C and 60 ± 5% relative humidity. Water was available at all times, with standard rodent chow (Special Diet Services, UK) freely available except from ZT7 to ZT12. Animals were allowed to recover for 2 weeks before the first drug administration.
Ola (ChemPacific Corp, Baltimore, USA) was solubilized in a minimum quantity of hydrochloric acid and diluted with purified water to the required concentration, after which pH was set at ~ 5.5 with 1M NaOH. All injections were administered 30 min before the beginning of the dark phase and in a counter-balanced order. On the first two test days, rats received IP injection of saline or Ola (1mg/kg). On two other test-days, rats received ICV injections of saline or 60 µg of Ola in 3 µl of saline (infused in ~ 1 min). All test-days were at least 3 days apart. Locomotor activity was automatically recorded by receiver plates below the home cage every 10 minutes using DSI software (Data Science International, St. Paul, Minnesota, USA). At the end of the experiment, placement of the ICV cannula was checked by injection of a methylene blue dye 10 minutes before animals were sacrificed. In addition, 30 minutes before sacrifice, rats received either an injection of Ola via their ICV cannula (60 µg, n=8) or an IP injection (1 mg/kg, n=2). After decapitation, trunk blood was collected in lithium-heparin coated tubes and brains were removed and dissected. Placement of the cannula was considered correct if dye was visible in both the lateral ventricle as well as the 4th ventricle. After centrifugation, plasma samples were stored at -20 ºC until HPLC analysis was performed to determine Ola concentrations. Statistical analysis was performed using SPSS version 20.0. To determine effects on locomotor activity, repeated measures ANOVA was performed, followed by paired samples T-test where appropriate. All experimental procedures were approved by the Committee for Animal Experimentation of Utrecht University and were conducted in agreement with Dutch laws (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

**Experiment 2**

A week after arrival, seven naive male Wistar rats (300 g) were equipped with either ICV cannula aimed at the lateral ventricle (n=3) or a silicon cannula in the stomach (n=4). Surgery was performed under fentanyl/ fluanisone (Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium) and midazolam (Dormicum®, Roche, Woerden, The Netherlands) anesthesia. ICV cannulas were placed into the lateral cerebral ventricle using a standard Kopf stereotaxic apparatus (Anteroposterior: -0.8mm; Lateral: 2.0mm; Ventral: -3.2mm; Angle=0). Intragastric (IG) cannulas were placed through a 1 cm incision on the left side of the stomach and tunnelled subcutaneously to the skull. IG or ICV cannulas were fixed on the skull using dental cement. Animals were then individually housed (40 x 25 x 25 cm plexiglas cages with wood shaving as bedding) in a specific pathogen free facility and maintained on a 12:12 light/dark cycle (lights on at 7:00 a.m.) at 21 ± 1°C and 60 ± 5% relative humidity, and given 7-10 days to recover from the surgery. Standard rodent chow (Teklad Global Diets) and water were available ad libitum. Ola solutions were prepared by dissolving Ola (ChemPacific
Corporation, Baltimore, U.S.A.) in purified water acidified with hydrochloric acid, brought back to pH ~6 using NaOH.

The peripheral group was treated with an IG infusion of 36 mg/kg/h for 5 minutes followed by a continuous infusion of 3 mg/kg/h for 160 minutes (i.e., in total ~3.66 mg Ola per rat). The central group was treated with an ICV infusion of 360 µg/kg/h for 5 minutes and 30 µg/kg/h for 160 minutes (i.e. in total 36.6 µg Ola per rat, representing 1% of the dose of the peripheral group). Infusions took place between ZT6 and ZT10.

At the end of the Ola infusion, rats received a lethal injection of pentobarbital, after which brains were collected in 2 ways: either collected fresh and frozen at -20ºC, or perfused with PBS (to get rid of any Ola-containing blood that remained in the cerebral vessels) and then collected and frozen -20ºC.

Brain Ola concentrations were measured with a calibration curve based on full brain homogenates by LC/MS/MS coupled to Quattro I Xe (Laboratoires Fournier, Solvay Pharmaceuticals now Abbot, Daix, France). All experimental procedures were approved by the animal care committee of the Royal Netherlands Academy of Arts and Science and were conducted in agreement with Dutch laws (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

RESULTS (part 2)

Experiment 1

Unfortunately, data of three rats had to be excluded due to misplaced ICV cannulas, and of two rats because of technical problems. To enable direct comparison of the effects of IP injection to ICV injection, only the data of the four remaining rats are presented in Figure 3. After IP injection, locomotor activity was clearly reduced by Ola treatment (Figure 3). Repeated measures ANOVA of the 12 hours following injection revealed a significant effect of treatment ($F_{(1,3)}=24.9$, $p=0.015$). Paired samples t-test revealed a significant effect on locomotor activity at ZT13 and a trend at ZT14 ($p=0.03$ and $p=0.073$ respectively).

Conversely, after ICV administration of Ola, locomotor activity was not significantly affected (Figure 3; ANOVA $F_{(1,3)}=0.04$, $p=0.95$).

Table 3 shows the concentration of Ola in plasma as determined by HPLC analysis. Data is shown for the two rats that had received an IP injection and for the four rats with correctly placed cannulas that had received an ICV injection. The results show that, after ICV injection, Ola was indeed detectable in plasma, indicating leakage into the circulation.
Figure 3: Locomotor activity of rats that received an injection of Ola intraperitoneally (A) and into the lateral ventricle (B). Arrows indicate the time of injection. Data is expressed as Mean ± S.E.M. *p<0.05, ~p=0.073

<table>
<thead>
<tr>
<th></th>
<th>Dose (µg/rat)</th>
<th>Plasma level (ng/ml)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>480</td>
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<td></td>
<td>142.5</td>
</tr>
<tr>
<td>450</td>
<td>140</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ICV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>38</td>
<td></td>
<td>35.5</td>
</tr>
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<td>30</td>
<td></td>
<td></td>
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<tr>
<td>60</td>
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<td></td>
</tr>
<tr>
<td>60</td>
<td>53</td>
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</tbody>
</table>

Table 3: Plasma Ola levels after IP or ICV infusions.
Moreover, plasma levels after ICV administration of 60 μg of Ola (which was ~13% of the peripherally administered dose) were approximately 25% of the levels observed after peripheral administration. Therefore, plasma levels seemed considerably higher than would be expected after ICV administration of this dose.

Experiment 2
Table 4 shows brain concentrations of Ola after IG vs. ICV administration. It shows that brain levels after peripheral administration were at least 20-fold higher than after central administration of Ola.

<table>
<thead>
<tr>
<th></th>
<th>Dose (μg/rat)</th>
<th>PBS rinsed</th>
<th>Brain level (ng/g)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>IG</td>
<td>3660</td>
<td>No</td>
<td>2531,5</td>
<td>2406</td>
</tr>
<tr>
<td></td>
<td>3660</td>
<td>No</td>
<td>2281,5</td>
<td>1484</td>
</tr>
<tr>
<td></td>
<td>3660</td>
<td>Yes</td>
<td>1544</td>
<td></td>
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<tr>
<td></td>
<td>3660</td>
<td>Yes</td>
<td>1424,6</td>
<td></td>
</tr>
<tr>
<td>ICV</td>
<td>36.6</td>
<td>No</td>
<td>28</td>
<td>44.8</td>
</tr>
<tr>
<td></td>
<td>36.6</td>
<td>No</td>
<td>61,6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>36.6</td>
<td>Yes</td>
<td>0,9</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Brain levels of Ola after IG and ICV infusion in Experiment 2.

DISCUSSION (part 2)
When Ola was administered acutely via an IP injection in Experiment 1, locomotor activity was clearly reduced during the first hours after injection, which was in line with previous findings (van der Zwaal et al. 2012). In contrast, we failed to observe an effect on locomotor activity after acute ICV administration in this experiment. Similarly, we previously observed effects of Ola on glucose metabolism after IG administration, but not ICV administration (Girault et al. 2012), suggesting that both of these effects are not primarily centrally mediated.

However, plasma Ola levels of ICV-treated rats in Experiment 1 were much higher than expected (Table 3), and Ola concentrations were higher than the detection threshold in over half of the animals receiving an ICV injection in the experiment investigating glucose metabolism, indicating leakage of Ola into the circulation. Moreover, in Experiment 2, brain
levels of Ola after ICV administration were much lower than expected, varying from 0.9-61.6 ng/g, compared to 1424.6-2531.5 ng/g for IG administration (Table 4). The concentration of Ola in brain tissue after ICV infusion was therefore only a fraction of that obtained after IG infusion (~1%). As the dose administered centrally was 1% of the dose administered peripherally, brain concentrations of Ola appeared to be approximately in proportion to the administered dose and independent of the route of administration. Taken together, these findings strongly suggest that, when Ola was infused into the lateral ventricle, it did not penetrate into the brain tissue very well, but was mostly absorbed into the circulation instead.

This seems remarkable, because Ola is a lipophilic drug that passes the blood-brain barrier very well (Aravagiri et al. 1999). Nevertheless, to our knowledge, it is unknown whether Ola passes the cerebrospinal fluid (CSF)-brain barrier with similar ease. It has previously been shown that marked differences exist in the ability of different compounds to pass the ependymal layer (which separates the ventricular system from the brain) and the distance that compounds can diffuse into the brain parenchyma after ICV administration. These differences even exist between compounds with similar molecular weight and also between different brain regions (Maness et al. 1996). Furthermore, after ICV administration of radioactively labeled compounds into the lateral ventricle, peak concentrations have already passed through the lateral and 3rd ventricles into the 4th ventricle within the first 5 minutes (Nagaraja et al. 2005). Any labeled compound that did not diffuse into brain tissue surrounding the ventricles, rapidly enters the subarachnoid space and basal cisterns which are rich in blood vessels, and although from here some diffusion into brain tissue is possible, a large part of the compound is absorbed into the circulation without having entered any brain tissue (Ghersi-Egea et al. 1996; Nagaraja et al. 2005). The time it takes for 50% of an ICV-administered compound to be cleared from the brain can range from 8 to 60 minutes and conversely, the appearance of an ICV-administered compound in the peripheral circulation can occur as early as 5 minutes after injection (Ghersi-Egea et al. 1996; Nagaraja et al. 2005). If Ola indeed showed poor penetration into the brain parenchyma after ICV administration, it is likely that a large part was similarly removed very rapidly from the brain and entered the general circulation, thereby resulting in surprisingly high plasma levels. If it was the case that all of the 60 µg of ICV-administered Ola in Experiment 1 initially “bypassed” the brain and entered the general circulation, this would be equivalent to ~13% of the dose administered per IP injection. Presuming that plasma levels after a single dose correlate roughly with the administered dose (Aravagiri et al. 1999; Callaghan et al. 1999; Citrome et al. 2009), plasma levels would be expected to be ~13% of those observed after IP injection, however plasma levels after ICV administration were nearly twice as high as expected (~25% of those after IP injection). Most likely, this is due to the marked clearance

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of IP-administered Ola by the liver, resulting in a large “first-pass” effect and lower plasma levels than after, for instance, subcutaneous administration (Choi et al. 2007; Kassahun et al. 1997).

In the study determining the effects of Ola on glucose metabolism (Experiment 2), based on the administered dose, plasma levels after 3 hours ICV-infusion of Ola (total dose 36.6 µg) would have been expected to be approximately 60% of the levels observed after administration of dose (60 µg) in Experiment 1 (Girault et al. 2012). However, levels measured after ICV administration were below the detection limit in 5 out of 12 rats. Most likely, this is due to the fact that the infusion took place over 3 hours, instead of via an acute injection. The half-life of Ola is shortest in plasma, as compared to tissues (e.g. brain, liver, kidney, lung), whereas the time to peak concentration is similar (~30 minutes) (Aravagiri et al. 1999). Thus, with prolonged infusion, Ola is likely to accumulate in tissues with longer half-lifes, resulting in much lower plasma levels than after acute injection of the same dose.

It has previously been suggested that the effects of Ola on glucose kinetics are due to effects directly on the hypothalamus, because effects after ICV administration were similar to those observed after intravenous administration of Ola (Martins et al. 2010). In this study, a total of 4.5 mg/kg Ola was delivered intravenously (~1.5 mg/rat) whereas a total of 330 µg/rat were delivered ICV over a period of 4 hours. Thus, the centrally administered dose was approximately 22% of the peripherally administered dose and more than 5 times higher than the dose administered in Experiment 1 (described above). As we already observed leakage of centrally administered Ola into the circulation after an ICV dose of 60 µg of Ola, it is likely that considerable leakage into the circulation occurred after ICV administration of 330 µg of Ola. Moreover, the centrally administered dose of 330 µg even approached the dose that was administered via a regular IP injection in Experiment 1 (1 mg/kg). It is therefore conceivable that the effects on glucose kinetics that were observed after ICV administration were still (in part) due to peripheral effects that occurred after leakage of Ola into the circulation. To our knowledge, the authors did not determine the concentration of Ola in plasma after the different modes of administration, and therefore it remains to be determined whether the effects they observed were indeed solely due to effects of Ola in the brain. Conversely, one cannot fully exclude central effects based on the absence of an effect of ICV-administered Ola as it appears to show poor penetration into brain tissue after ICV administration.

Because regular ICV injections of Ola apparently do not result in effective administration into the brain, a different approach is necessary to enable investigation of the centrally mediated effects of this drug. Administration directly into the brain tissue could be performed via an implanted cannula or microdialysis probe (Diaz-Mataix et al. 2005). When a compound is
delivered directly into brain tissue, its removal from the brain is slower than after ICV injection, because clearance into the circulation only occurs via the intraparenchymal veins (Nagaraja et al. 2005). Furthermore, the required dose would be much lower, and the effect on plasma levels would therefore be negligible. A disadvantage of this technique, however, is that it is only possible to target one brain area at a time, and it depends on the diffusion rate of Ola into the brain parenchyma how large the targeted area will actually be. Moreover, as Ola is a compound with a large structure, special care would have to be taken regarding the choice of the membrane permeability in case of an experiment using a microdialysis probe.

Another option to deliver Ola selectively into the brain would be to equip animals with an intra-carotid cannula directed to the brain (Cruciani-Guglielmacci et al. 2004), which would enable fairly selective infusion into most parts of the brain. A disadvantage of this technique is that some leakage into the peripheral circulation will still occur, although this can be controlled for by administering the same dose intravenously.

Finally, it may be possible to administer Ola solution effectively via an ICV cannula if it contains an adjuvant that facilitates its absorption into the brain, i.e. DMSO. However, ICV administration of adjuvants may cause effects on their own, which would need to be controlled for (Nasrallah et al. 2008). In case the adjuvant causes a similar effect as Ola, a ‘floor’ or ‘ceiling’ effect could thereby mask effects of Ola itself. Furthermore, when Ola is peripherally administered and enters the brain via the circulation it distributes fairly evenly throughout the brain (Aravagiri et al. 1999). However, after administration of radioactively labeled compounds into the lateral ventricle, distribution of the compound is unequal for different brain regions and only traces of radioactivity are observed e.g. in the CSF surrounding the cerebral cortex (Ghersi-Egea et al. 1996). Therefore, even if an adjuvant is used to promote brain penetration, it is still possible that ICV-administered Ola ‘misses’ certain brain areas that might be involved in its effects on energy balance, i.e. (sub)cortical structures.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICV injection of Ola</td>
<td>Easy</td>
<td>Poor penetration into brain tissue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leakage into peripheral circulation</td>
</tr>
<tr>
<td>ICV injection of Ola using adjuvant to</td>
<td>Easy</td>
<td>Adjuvants may have effects of their own</td>
</tr>
<tr>
<td>facilitate absorption</td>
<td></td>
<td>Not likely to reach all brain areas</td>
</tr>
<tr>
<td>Local Ola injection in the brain</td>
<td>Better brain penetration</td>
<td>Can target only separate/small brain areas</td>
</tr>
<tr>
<td>Intracerebral microdialysis probe</td>
<td>Low risk of tissue damage</td>
<td>Can target only separate/small brain areas</td>
</tr>
<tr>
<td>administration of Ola</td>
<td></td>
<td>High costs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Requires adequate permeability of the membrane for Ola</td>
</tr>
<tr>
<td>Intra-carotid infusion of Ola</td>
<td>Good brain penetration</td>
<td>Leakage into peripheral circulation</td>
</tr>
<tr>
<td></td>
<td>Targets most of the brain</td>
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</table>

Table 5: Summary of advantages and disadvantages of different modes of central administration of Ola.
Taken together, although ICV administration of a regular solution of Ola does not seem an effective way to investigate the central effects of Ola, several different approaches remain (summarized in table 5).

CONCLUSIONS

The experiments described in this paper clearly indicate that several factors can complicate the administration of Ola in a rat model. Due to the short half-life of Ola in rats, simple daily injections would not result in chronic drug exposure that is comparable to humans. Circumventing this issue by using implantable osmotic minipumps is problematic due to degradation of Ola in an acidified aqueous solution at body temperature, whereas administration via the drinking water has secondary effects on water intake due to the bitter taste. Finally, central administration of Ola appears challenging, due to poor penetration into brain tissue and leakage of Ola into the general circulation after ICV infusion.

The main goal of this paper was to raise awareness of the technical issues concerning administration of Ola in a rat model and to hopefully contribute to the development of future animal models investigating Ola-induced weight gain. However, similar issues may arise with the administration of other drugs as well. During the development of new models investigating drug effects in rodents, such issues can have serious consequences for the interpretation of results. Therefore, this paper also illustrates the importance of verifying whether a chosen mode of drug administration is actually effective, before embarking on large-scale experiments.

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Reference List


humans. *Drug Metabolism and Disposition* **25**: 81-93.


the treatment of acute mania: a systematic review and meta-analysis of randomized controlled trials. *Arch Gen Psychiatry* **64**: 442-455.


