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Micropipette-guided Drug Administration (MDA) as a non-invasive chronic oral administration method in male rats

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ABSTRACT

Background: In preclinical studies resorting to rodents, the effects of prolonged oral intake of active substances are difficult to evaluate. Indeed, to get closer to clinical reality, oral gavage (OG) is frequently used but the repetition of administrations induces risks of lesions of the digestive tract, and stress for animals which can compromise the quality of the results.
New method: This study describes the development of a non-invasive oral administration method in male Sprague Dawley rats, as a safe alternative of OG, more faithful to clinical reality and limiting biases in pharmacokinetics and/or pharmacodynamics interpretation. Micropipette-guided Drug Administration (MDA) is based on the administration by micropipette of a sufficiently palatable vehicle for the animals to voluntarily take its contents.
Results: MDA was not demonstrated as less stressful than OG. A pharmacokinetics equivalence between MDA and OG was demonstrated for pregabalin administration but not for aripiprazole. Despite the use of a sweet vehicle, the MDA method does not result in weight gain or significant elevation of blood glucose and fructosamines level. Regarding the time needed to administrate the solution, the MDA method is significantly faster than OG.
Comparison with existing method(s): Contrastingly to procedures using food or water, this method allows for a rigorous control of the time and dose administered and is delivered in discrete administration windows which is therefore closer to the clinical reality. This method appears particularly suitable for pharmacological evaluation of hydrophilic compounds.
Conclusions: The MDA procedure represents a respectful and adapted pharmacological administration method to study the effects of chronic oral administration in rats.

1. Introduction

Drug consumption is associated with a risk of adverse effects, the probability of which varies according to patient (age, sex, weight, *etc.*) and the progression of the pathology. Prior to marketing, drug adverse effects are identified during clinical trials. As a result, a specific warning is given to patient during prescription to enable detection of adverse effects as early as possible. Unfortunately, some adverse effects may go undetected during clinical trials. The duration of exposure investigated is too short (relative to a lifelong treatment administered for chronic disease) to identify late-onset adverse effects. Post-marketing pharmacovigilance systems and real-life pharmaco-epidemiological studies may identify some associations between a given drug or a drug class and an adverse effect. However, this new signal needs to be confirmed and studied by further investigations. Animal models could help to better understand the reasons for late-onset adverse effects and thus improve our drug prescription practices and make them safer.

Oral route is the preferred method of drug administration (80% of pharmaceutical forms distributed), mainly because it is less invasive and less expensive. In the field of preclinical research on rodents, oral gavage (OG) is hence used to promote the transition to clinical use. However, while OG technique has the advantage of precise control over time and dose of administration, it also does have drawbacks. Indeed, a certain degree of technical skills is required from experimenters, since the

Abbreviations: MDA, Micropipette-guided Drug Administration.

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animal has to be restrained. In addition to discomfort, restraint can be a source of stress for animals (Stuart and Robinson, 2015). Beyond all this, repeated OG can also lead to aspiration pneumonia, as well as irritation, injury or even rupture of pharyngeal, esophageal and/or gastric membranes (Brown et al., 2000).

In line with evolving ethical concerns and in order to respect animal welfare as much as possible, new less invasive oral administration techniques have been developed. One such strategy consists in adding the drug either to the feed (Abelson et al., 2012; Diogo et al., 2015; Hovard et al., 2015) or to the drinking water (Zhan et al., 2019). Although these are stress-free methods for animals as no interaction is required, animals need to be housed individually to ensure correct dosing. Ideally, rats should be housed in groups to reduce the stress levels (Sharp et al., 2002). They might also be criticized from a pharmacological point of view. Indeed, constant access to a pharmacological substance (*via* food or water) could lead to a different plasmatic profile to a clinical situation with oral administration at specific time intervals (Turner et al., 2011a,2011b).

Recently, an innovative non-invasive method called Micropipette-guided Drug Administration* (MDA) has been described in the mouse (Scarborough et al., 2020). This method takes advantage of the sweet and appetizing properties of sweetened condensed milk, which is used as a vehicle for drug delivery. Its palatability induces voluntary ingestion of the micropipette solution by the animals. Taking into account ethical concerns, this method also appears to be more comparable to oral administration used in humans. Authors also demonstrated that MDA was less stressful for mice than OG and intraperitoneal (IP) administration (respectively Scarborough et al., 2020; Schalbetter et al., 2021). However, the effect of using a sweet vehicle (often criticized) on glucose metabolism has never been evaluated. Techniques using this type of delivery, such as those using syringes, are often criticized for being very time-consuming (Atcha et al., 2010). For the first time, we compared the time required for the administration procedure by OG *vs* MDA.

Herein, we aimed to adapt this method for use in rats. Rats have a wider behavioral repertoire than mice and have a higher blood volume, which is sometimes necessary when performing numerous biochemical assays. The practicality / compatibility of the method was evaluated and compared to the commonly used oral gavage (OG) method. This included assessment of animals motivation / willingness over-time (over repeated administrations), animal welfare (corticosterone, glucose and fructosamine levels). Furthermore, a comparative study of the pharmacokinetic properties of the two methods (MDA *versus* OG) was performed using two psychoactive substances. For this purpose, the antiepileptic drug pregabalin and the antipsychotic drug aripiprazole were chosen. These are two widely used drugs prescribed for long-term illness(Evoy et al., 2021; Rhee et al., 2020). They are of particular interest for such pharmacokinetic studies because of their different physicochemical properties.

2. Materials and methods

2.1. Ethical concerns

Experimental procedures were performed in accordance with the European Communities Council Directive (2010/63/UE) and approved by the regional ethical committee (Comité d'Ethique NOrmandie en Matière d'EXpérimentation Animale, CENOMEXA) (agreement number: 35428).

2.2. Animals

Male Sprague-Dawley rats (n = 45), 6 weeks of age at the start of the protocol were used (Janvier Labs, France). Animals were randomly housed in pairs in standard polyacrylic cages (42x26x18,5 cm^3) with free access to food and tap water. Cage enrichment consisted in crinkle cut shredded paper and a cardboard roll. The animal facility was maintained under a normal 12 h light-dark cycle (lights on from 7 am to 7 pm), with a constant temperature (22 ± 1 °C) and humidity (50 ± 10%). During the first week following arrival, rats were left undistributed (acclimation to animal facility). During the second week, daily handling sessions were performed to accustom the rats to the experimenter (5 min per day, 7 days).

2.3. Oral administration methods and general experimental design

Random tables were used to separate animals into 2 groups according to the oral administration method: OG (n = 22) or MDA (n = 23) (see Fig. 1). Treatment (either OG or MDA method) was performed daily (early in the morning) for 3 weeks (from D1 to D21) in the administration room separate from the animal facility. Of note, to avoid additional stress, animals were acclimated in the administration room for 30 min

OG group: the procedure began on D1. Briefly, rats were restrained by the trunk (see Fig. 2 A) and a probe was inserted into the mouth all way down to the stomach. Of note, a flexible plastic probe (PHYMEP®) was used for gavage refinement (Ökva and Tamos, 2006). In addition, to limit mortality or adverse respiratory effects, the gavage volume was set at 1.5 mL/kg.

MDA group: a two-phase training procedure is required. As a one-week procedure, this training can be performed during the animal's handling phase to save time (as we did here) (see Fig. 1). During the first 3 days, animals were habituated to the smell and taste of a sweetened condensed milk solution (RÉGILAIT® full-fat milk: saccharose 45%, fat 8%, defatted lactic dry extract 20%), diluted at 3/10 with rodent bottle water. A 2 mL cup of this appetizing solution was placed in the cage each day. On the last four days of the week, a micropipette P1000 tip (Starlab®) containing the solution was presented to the rats through the cage grid. The pipette was left in place until the rat licked the tip and swallowed the solution (see Fig. 2B and Supplementary material – Video). The administration volume was set at 0.5 mL/kg. If an animal refuses to consume the micropipette content, a gentle restraint can be made for administration.

2.4. Effects of the administration method on the animal welfare

In each group (OG and MDA), 14 rats were used. They were treated daily for a chronic period (3weeks) (Fig. 3).

2.4.1. Body weight evolution

On each morning of the experimental protocol, just prior to administration, all animals in these groups (n = 28) were weighted from D-6 to D21.

2.4.2. Behavioral assessment

All devices were cleaned with 70% ethanol at the beginning and between each animal to avoid odor cues. Behavioral tests were performed 1 h after oral administration.

On D1, *i.e.* after the first oral administration, an elevated plus maze test was performed. The polyvinyl chloride apparatus consisted of 4 arms (50x10cm^2), with a central platform (10x10cm^2; Imetronic®). Two opposite arms were open (40Lux) and other two were closed by a 30 cm high wall (10Lux). The apparatus was elevated 75 cm above the floor. A rat was placed on the central platform facing an open arm and allowed to explore the apparatus for 5 min. The number of entries and the percentage of time spent in the open arms were calculated and used as an index of anxiety-like behavior (Lister, 1987).

On D20, *i.e.* after 3 weeks of daily administration, an open field test was performed. The apparatus consisted of a white polyvinyl chloride open box (100x100x45cm^3, 10Lux at the center, 7Lux at the corners). The rat was placed in the center of the apparatus and allowed to explore freely for 20 min (Soares-Silva et al., 2022). The percentage of time spent in the center was calculated and used as an index of anxiety-like

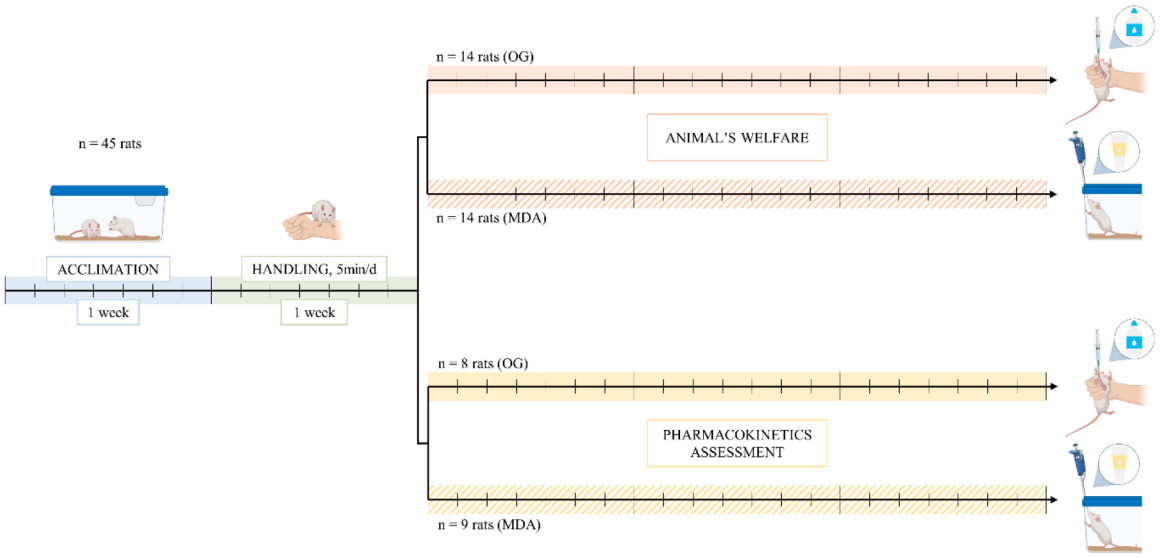

Fig. 1. General experiment design (created in BioRender.com).

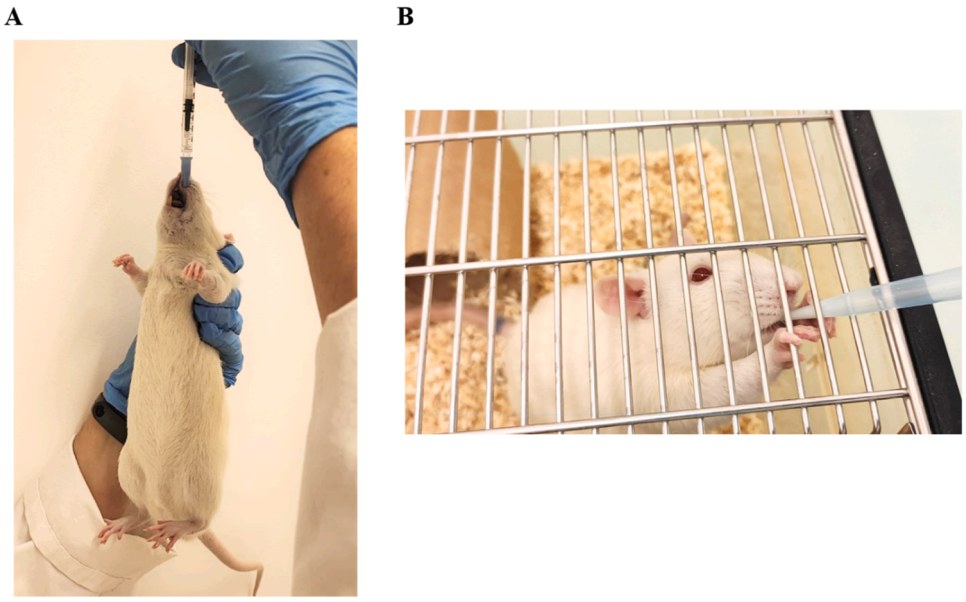

Fig. 2. (**A**) Restraint of the rat during OG method, (**B**) Micropipette displays through cage grid during MDA method.

behavior.

2.4.3. Biochemical analysis

To compare both acute and chronic effects of OG *versus* MDA, blood samples were collected at two time points of the treatment procedure, either 30 min after a single administration (n = 4) or after 3 weeks of daily treatment (n = 10). Rats received an intraperitoneal bolus of pentobarbital (120 mg/kg in a volume of 2,19 mL/kg) and were killed by cervical dislocation 5 min later. Blood was then collected from the jugular vein in either 2 mL sodium fluoride or 4 mL sodium heparin tubes. Blood collected in sodium fluoride tubes was centrifuged (3000 g, 10 min, Room Temperature) and the supernatant plasma was collected and stored at − 20 °C until analysis. Glycemia was measured through glucose hexokinase method (DxC 700 AU, Beckman-Coulter Instruments, USA). Blood collected on sodium heparin tubes was centrifuged (3000 g, 20 min, 4 °C) and the supernatant plasma was collected and stored at − 20 °C until analysis. Fructosamine levels were determined through colorimetric method (Konelab, Thermo Fisher Diagnostic). Corticosterone concentration was measured as a stress indicator (Marin et al., 2007) using liquid chromatography/tandem mass spectrometry (LC-MS/MS). Chromatography was performed on an ABSciex API 5500 QTRAP triple quadrupole mass spectrometer (Framingham, MA USA) equipped with an electrospray ionization source.

2.5. Effects of the administration methods on the pharmacokinetics

Two drugs, pregabalin and aripiprazole (see below; n = 4 rats per group, except for MDA-aripiprazole with n = 5 rats) were studied to compare the two methods of oral administration (OG *versus* MDA) from a pharmacological point of view (see Fig. 4).

2.5.1. Pharmacological agents

The two psychotropic drugs were chosen because of their different physicochemical properties: pregabalin (Merck®) and aripiprazole (Thermo Fisher®). The former, pregabalin, is a hydrophilic compound (logP=−1.78), while the latter, aripiprazole, is a lipophilic compound

Video S1. Administration process of Micropipette-guided Drug Administration.A video clip is available online. Supplementary material related to this article can be found online at doi:10.1016/j.jneumeth.2023.109951.

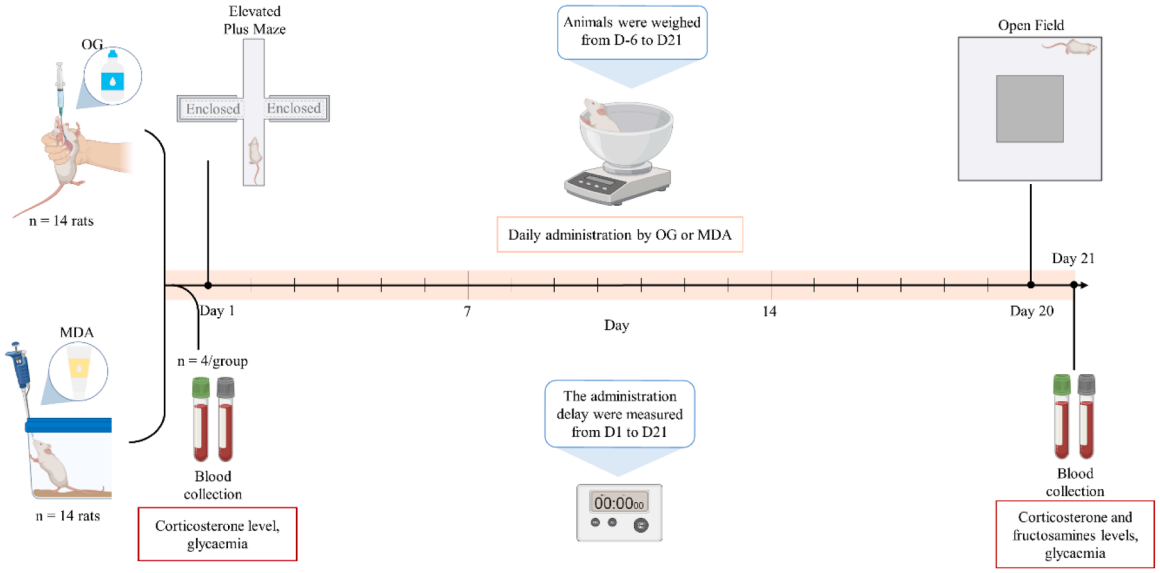

Fig. 3. Experimental protocol of effects of the administration method on the animal welfare *(created in BioRender.com)*.

(logP=5.30, pKa_1 =7.46, pKa_2 =13.51). Doses of pregabalin and aripiprazole (10 and 3 mg/kg, respectively) were chosen according to literature pharmacokinetics data (Lau et al., 2013; Raish et al., 2019). Considering its physicochemical properties, aripiprazole was first prepared in a mixed of Tween 20 (1% solution (Martins et al., 2008)) and acetic acid (0.17‰ solution), and finally in the vehicle solution (pH=6.18). Vehicle solution was either diluted in sweetened milk or in saline, according to oral methods used (MDA and OG, respectively).

2.5.2. Pharmacokinetic studies

At the end of the 3-weeks treatment period, rats were sacrificed by cranial percussion and decapitation one hour after the last administration. This killing method was used because it does not need any sedation and thus avoids any drug interaction with the anesthetic. Blood was collected directly from the jugular vein into sodium heparin tubes (4 mL capacity). Samples were centrifuged (3000 g, 20 min, RT) and the supernatant plasma was then collected and stored at − 20 °C until analysis.

Pregabalin and aripiprazole plasma concentrations were measured using liquid chromatography/tandem mass spectrometry (LC-MS/MS) according to an adaptation of the analytical conditions described by Zhang et al. (2022) and Kirschbaum et al. (2010) respectively. Chromatography was performed on an ABSciex API 4500 QTRAP triple quadrupole mass spectrometer (Framingham, MA, USA) equipped with an electrospray ionization source. Acquisition was realized in multiple reaction monitoring (MRM) mode. Separation was performed on a

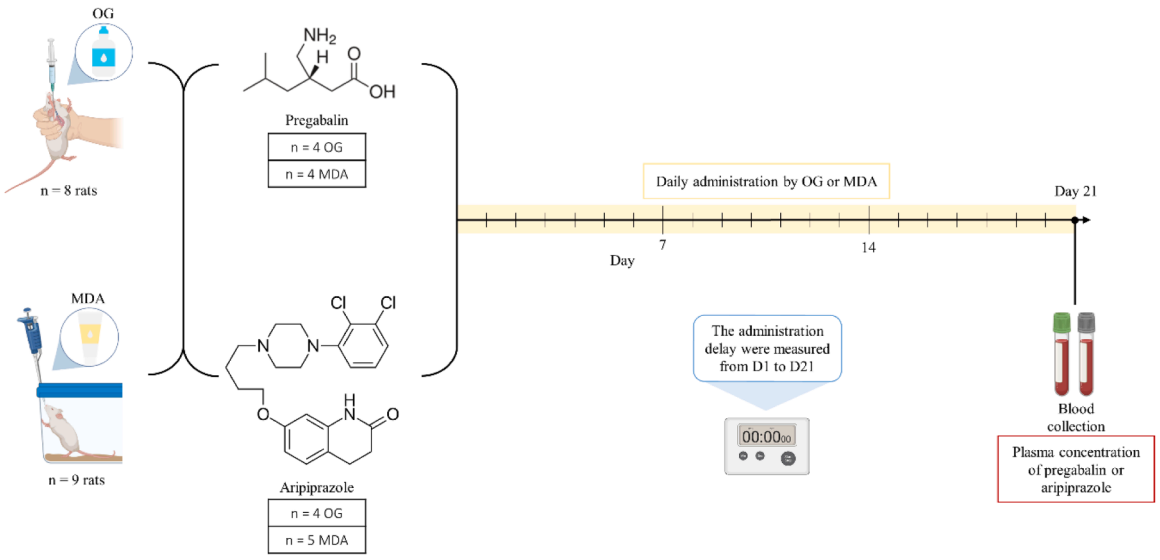

Fig. 4. Experimental protocol of assessment of pharmacokinetics equivalence *(created in BioRender.com)*.

reversed-phase Biphenyl column (2.1x100mm, Phenomenex, USA). Mobile phases consisted of 1 mM ammonium formate in water and 0.02% formic acid, (A phase) and ammonium formate 1 mM in methanol and 0.02% formic acid (B phase), with gradient elution at 0.5 mL/min. Column temperature was maintained at 40 °C. All analyses were performed using positive ion ESI (ESI+).

Pregabalin was extracted from rat plasma using protein precipitation. To 100 µL of plasma were added, 200 µL of internal standard (D6-Pregabalin, 0,5 mg/L) and 100 µL of water were added. The supernatant of the protein precipitated mixture was extracted and reconstituted in 200 mL of acetonitrile for a second centrifugation prior to LC-MS/MS quantification.

Aripiprazole was extracted from rat plasma using liquid-liquid extraction. To 100 µL of plasma were added 50 µL of internal standards (D6-Venlafaxine and D3-Olanzapine, 250 ng/mL), 500 µL of 0,25 M sodium hydroxide solution and a mixture of ethyl acetate/dichloromethane/hexane (40/22/38 v/v). The organic phase was extracted and dried, then the extract was reconstituted in 100 mL of A mobile phase prior to LC-MS/MS quantification.

2.6. Applicability of the administration method to large groups

The delay to perform oral administrations was daily collected from D1 to D21 for all animals (n = 45). This time includes the weighing of the rat, the preparation of either syringe or micropipette and the administration itself.

2.7. Statistical analysis and figures

Statistical analyses were performed using Prism (version 8.0; GraphPad Software, USA). Data that followed a Gaussian distribution (Kolmogorov-Smirnov normality test) and had equal variances are presented as mean ± Standard Error Mean (SEM) and were analyzed using parametric statistical tests (unpaired student t-test or ANOVA for repeated measures). Data sets that did not meet either of these criteria are presented as median ± interquartile range (IQ) and were analyzed using non-parametric statistical tests (Kolmogorov-Smirnov test). Statistical significance was set at $p < 0.05$.

Figures have been created with BioRender.com (agreement number: MG252P2KJK).

3. Results

3.1. Effects of the administration method on the animal welfare

3.1.1. Stress response

Anxiety-like behavior was first assessed by the EPM after a single oral administration, then by the OF test 3 weeks later after repetitive daily oral administration. No statistical group difference (OG *versus* MDA) was observed in the EPM test (Supplemental Fig. 1). Similarly, the OF test showed no major difference between groups. In fact, neither the total distance moved nor the percentage of time spent in the central zone differed between groups (Fig. 5A; $t(18) = 0.81$, $p = 0.43$; Fig. 5B; $t(18) = 0.75$, $p = 0.46$, unpaired t-test). Although, a statistically higher number of entries in the central zone was observed for MDA group, reflecting thus a lower level of anxiety-like behavior (Fig. 5C; $t(18) = 2.19$, $p = 0.04$, unpaired t-test).

In line with the behavioral results, plasma corticosterone levels did not statistically differ from one method to another (OG *versus* MDA), neither at D21 (Fig. 5D; $t(18) = 0.60$, $p = 0.56$, unpaired t-test), nor at D1 (Fig. 5E; D=0.50, $p = 0.77$, Kolmogorov-Smirnov test).

3.1.2. Impact of the use of a sweet vehicle on body weight and the carbohydrate metabolism

Analysis of body weight curve revealed a significant time effect ($F(27, 486) = 1254$, $p < 0.0001$), but neither group effect (OG *versus* MDA) ($F(1, 18) = 0.39$, $p = 0.54$), nor time x group interaction ($F(27, 486) = 0.78$, $p = 0.77$) (Fig. 6A). Thus, chronic daily consumption of the condensed milk solution required for MDA did not influence body weight gain in the MDA group (compared to the OG group).

Regarding carbohydrate metabolism, no significant group difference (OG *versus* MDA) was observed for blood glucose levels, either at D1 (Supplemental Fig. 3) or at D21 (Fig. 6B; $t(18) = 1.03$, $p = 0.32$, unpaired t-test). Besides, after 3 weeks of treatment, no group difference was found in plasma fructosamine levels at D21 (Fig. 6C; $t(18) = 0.14$, $p = 0.89$, unpaired t-test).

3.2. Effects of the administration method on the pharmacokinetics

Pregabalin plasma concentrations did not differ between groups (20.43 ± 2.26 ng/mL and 18.90 ± 0.95 ng/mL for OG and MDA, respectively) (Fig. 7A; D=0.25, $p > 0.99$, Kolmogorov-Smirnov test).

Conversely, a significant group difference was observed for aripiprazole plasma concentrations. The MDA group had a significantly

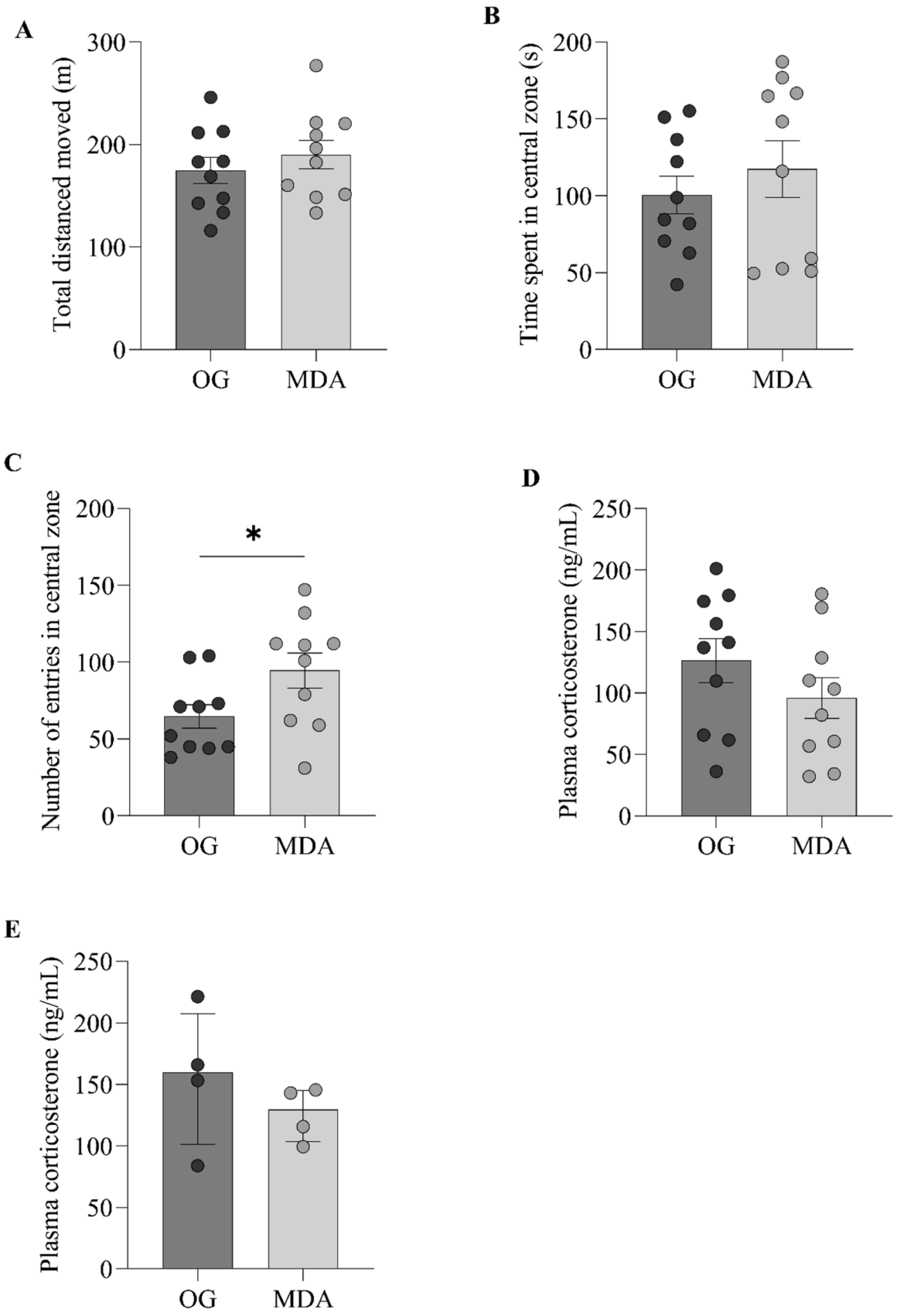

Fig. 5. Effects of 3 weeks daily oral administration (OG or MDA) on behavioral parameters in the open field test (**A**) Distance moved; (**B**) Time spent in central zone; (**C**) Number of entries in central zone; on (**D**) plasma corticosterone concentration; and on (**E**) plasma concentration after a single oral administration. *$p < 0.05$, based on unpaired t-test.

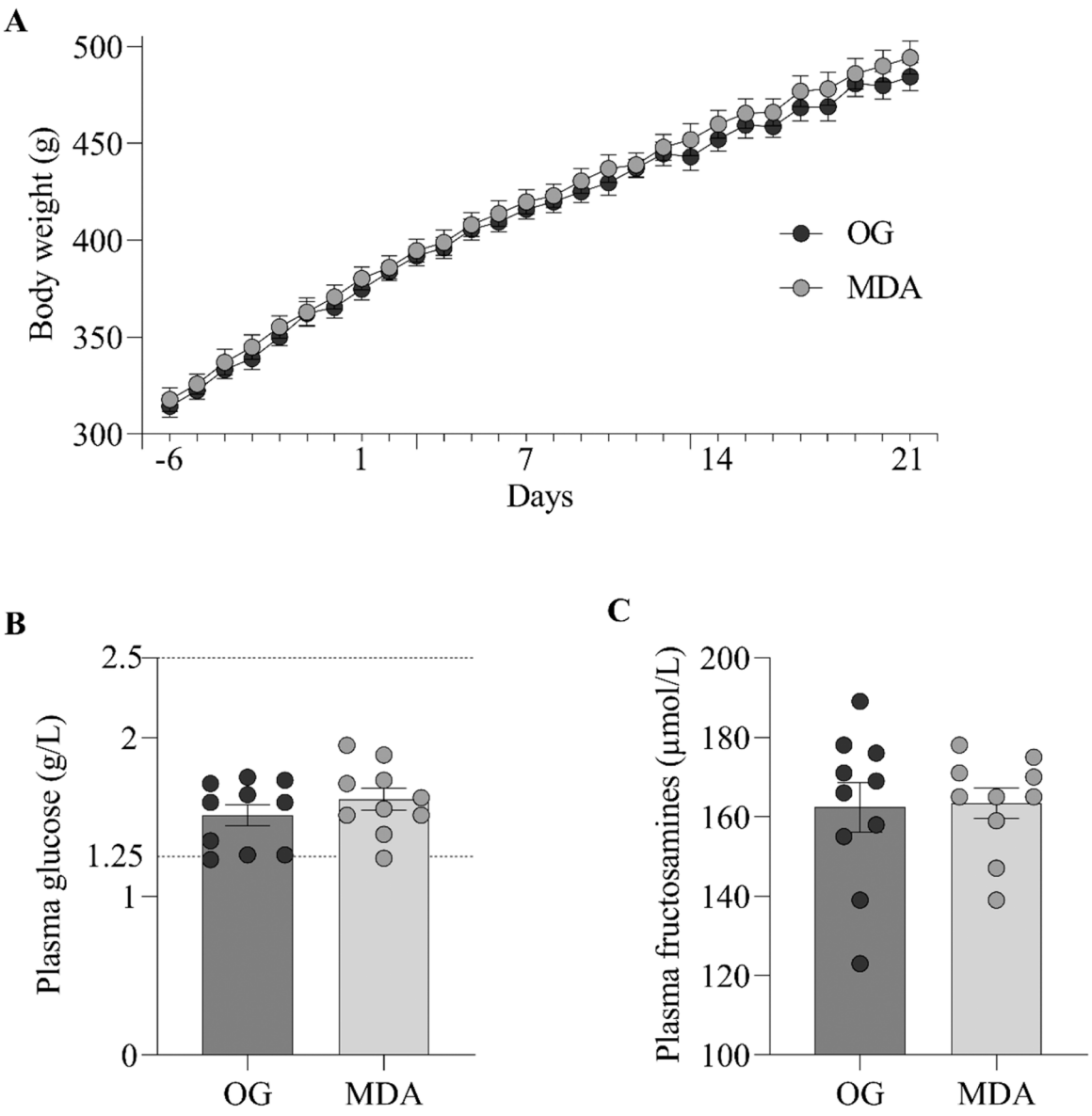

Fig. 6. Effects of 3 weeks daily oral administration (OG or MDA) on (**A**) body weight gain and serum concentration of (**B**) glucose and (**C**) fructosamines. Dotted lines reflect limits of normal values for serum glucose and fructosamines concentrations. ns=non-significant.

lower value than the OG group (4.59 ± 0.58 ng/mL and 11.55 ± 2.58 ng/mL, respectively) (Fig. 7B; D=1, p = 0.016, Kolmogorov-Smirnov test).

3.3. Applicability of the administration method to large groups

At the end of training, the average time for rats to consume the condensed milk solution from the micropipette by MDA method was 3.45 ± 0.13 s per rat. The time duration for the two procedures was measured and compared. A significant group difference was observed, with MDA being faster to realize (119.5 ± 8.35 s/rat and 68.71 ± 10.3 s/rat, for OG and MDA, respectively) (Fig. 8A; t(32.38) = 6.74, p < 0.0001, unpaired t-test). This difference may have important implications in the context of chronic dosing experiments. Analysis of the cumulative time of administration curve revealed a significant group effect (OG *versus* MDA) (F(1, 35)= 11.93, p = 0.0015), a significant time x group interaction (F(20, 700)= 13.03, p < 0.0001) and a significant time effect (F(1.05, 36.81)= 178.8, p < 0.0001) (Fig. 8B). In our hands, it has led to a gain of 5 h in favor of MDA methods for thus a 3 weeks daily administration of 18 (OG) and 19 (MDA) rats (in favor of MDA, respectively 12.55 h while MDA method lasted 7.62 h). Of note, no difference was observed according to the administration delay of pregabalin *versus* aripiprazole (Fig. 8C; t(40) = 0.59, p = 0.56, unpaired t-test). It should be noted that on two occasions (different animals in different days, *i.e.* D1 and D17), two rats (out of a total of 45) did not show any interest in the tip and thus required to be a slight restraint for administration when necessary.

4. Discussion

We described and characterized for the first time in rats, the Micropipette-guided Drug Administration (MDA) method. We have shown that this method can be adapted from mice to rats. Easy to perform and non-invasive and therefore in some way safer for the animals (compared to other existing methods), but also less time-consuming, this new oral administration method in rodents is also closer to the clinical reality. Obviously, this method appears even more valuable when oral administration has to be performed chronically.

Animal welfare was assessed after a single administration and after 3 weeks of daily administration. Quite surprisingly, no significant

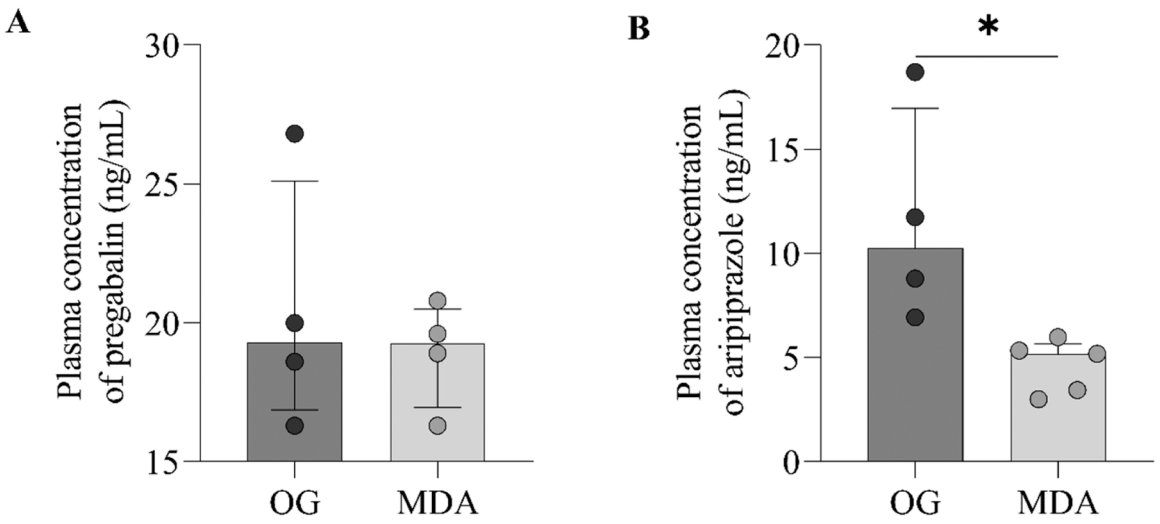

Fig. 7. Plasma concentration of (**A**) pregabalin (10 mg/kg) or (**B**) aripiprazole (3 mg/kg) administrated daily during 3 weeks, through either OG or MDA. Blood sample was realized 1 h after last administration. *$p < 0.05$, Kolmogorov-Smirnov test.

difference was observed between the two methods. In fact, both behavioral and biochemical results indicate a similar level of stress between the two methods (OG *versus* MDA). These results are unexpected as they contrast with literature data in the mouse model, where a lower level of corticosterone was observed in the MDA group compared to OG (Scarborough et al., 2020). One possible explanation could be the refinement we made of the OG method. First, in contrast to the paper in mouse, we used plastic probes for OG administration, thus minimizing animal stress (Wheatley, 2002). Second, we used an administration volume (1.5 mL/kg) well below the maximum limit (5 mL/kg) (Damsch et al., 2011), which *per se* also contributes to animal welfare. Besides, it is worth noting that for small administration volumes, the level of stress induced by the gavage is not higher than that of a simple contention (Turner et al., 2012). In addition, habituation to restraint may reduce the stress associated with the procedure (Turner et al., 2011a,2011b). This must have interfered with our data, as animal restraint was required either to place the animal in the behavioral apparatus or for animal killing before blood sample analysis (Stuart and Robinson, 2015). In particular, the killing method we used, which is quite stressful (Vahl et al., 2005), may have masked a difference in corticosterone levels between groups. In other words, the corticosterone level observed here may reflect more the stress induced by the euthanasia method rather than that due to the oral administration method used previously.

Beyond any biochemical and/or behavioral results, several arguments in favor of a higher level of anxiety in the OG group were noted and should be mentioned. First, home cages of the OG animals were dirtier than those of the MDA animals (more feces and urine). Moreover, the cardboard rolls provided as cage enrichment were chewed more by OG rats than by MDA rats. As a result, they had to be changed twice more often for OG group than MDA group. Finally, while MDA rats stopped defecating into the bowl used for daily weighing, OG rats did not (a persistent behavior that may be associated with a stressful situation relative to the subsequent contention after weighing required for oral administration).

The MDA method requires a sweetened solution, which has to be palatable enough to motivate rodents to voluntarily ingest the contents of the micropipette, or even to mask the potentially aversive taste of pharmacological compounds. Herein, we used dilute condensed milk solution as it was previously described for mice (Scarborough et al., 2020). Nevertheless, individual taste preferences may lead to palatability issues and behavioral changes over time in some animals that would not or no longer cooperate (Turner et al., 2011a,2011b). In our study, two animals did not fully acclimate to the MDA method and showed a higher consumption delay. Beyond compound palatability and/or individual taste preferences, the active substance tested may also influence the degree of adherence or cooperation of the rat to the method. For example, if the tested pharmacological compound causes rapid onset side effects, the animal may associate the oral treatment with the impending discomfort events. Whether the animals become less prone to lick and swallow the tip, the animal will then require special attention. Of note, this situation is obviously not specific to the MDA method but is related to the properties of pharmacological agents tested. Thus, such an event has already been described when administered in a sucrose solution (Atcha et al., 2010).

Since the MDA method uses a sweet vehicle, its effect was evaluated *by assessing* the animals' body weight, glycemia and fructosamine level. The evolution of the body weight as the rats treated with MDA did not differ with the OG group. The use of sweetened condensed milk has no long-term effects on body weight compared to saline. This result is consistent with the blood glucose levels, the administration of a sweetened vehicle did not affect the animals' glycemia. Glucose levels were variable because samples were not collected on an empty stomach. Fasting blood glucose should be approximately 1.27 g/L (Nowland et al., 2011) and breeding labs provide glycemia data specific to the Sprague Dawley strain: 1.5–1.7 g/L (Janvier Labs) and 2.5 g/L (Charles River). Since glycemia is an acute indicator, measuring fructosamine levels provided a reflection of glucose concentration over the last 3 weeks. As expected, no significant difference was found between the two groups and the obtained values are close to those reported in the literature (Ejdesjö et al., 2011). If these results support an absence of metabolic consequences associated with the use of a sweet vehicle, this may be a limitation in experiments using rewards.

A pharmacokinetic study was performed to investigate how much equivalent or different the OG and MDA methods are. Two pharmacological substances with different physicochemical properties were studied: aripiprazole and pregabalin.

For pregabalin study, similar plasma concentrations were observed after MDA and OG, suggesting a pharmacokinetic equivalence of the two administration methods for hydrophilic compounds. In addition, it is worth noting that we found similar concentrations that already reported using the IP method at 10 mg/kg (Lau et al., 2013).

Conversely, a statistical group difference (OG *versus* MDA) was observed in the plasma concentration of aripiprazole. Quite interestingly, the concentration we observed with the MDA method was consistent with literature data for oral administration (OG, 2.5 ng/mL after 3 mg/kg 15days of daily administration) (Raish et al., 2019).

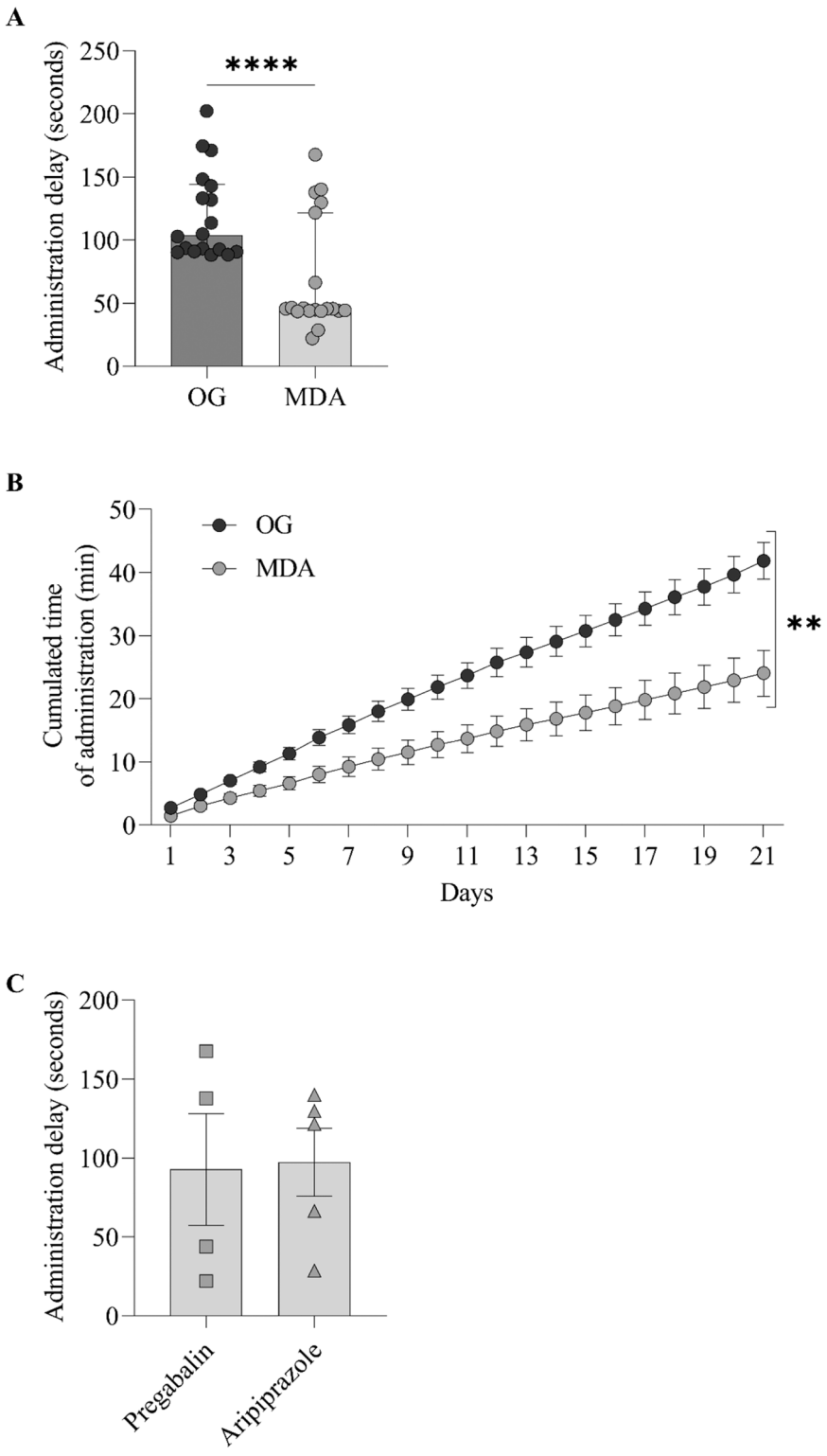

Fig. 8. Comparison of average time duration for each method (OG *versus* MDA). (**A**) Administration delay. (**C**) Cumulated time duration for administration during 3 weeks (**B**) MDA delay of pregabalin *versus* aripiprazole. * *p < 0.01, based on unpaired t-test. * ** *p < 0.0001, based on Kolmogorov-Smirnov test.

Therefore, this led us to search for an explanation for the unusually high level of plasma concentration of aripiprazole we found after OG. As suggested by the dispersion of the results observed, an issue of solubilization of the compound could explain our result. Indeed, the suspension variability of this hydrophobic compound could have affected the OG measurement. To verify this hypothesis, a sonication step could be added to the solubilization protocol of hydrophobic compounds in future experiments. Indeed, by using this method, Scarborough and colleagues demonstrated pharmacokinetic equivalence of OG and MDA for risperidone, a lipophilic compound (Scarborough et al., 2020).

One may discuss on the relevance of using the MDA method and qualify as a tedious method to perform. At a first sight, the MDA method appears to be more time-consuming than the classical OG method. In fact, one week of training prior to treatment is mandatory to accustom

the animal to the method and to overcome inter-individual preferences. However, when we looked at the time required for treatment in each group, it clearly appeared that MDA method is faster than OG one. Ultimately, the time spent before starting treatment can be quickly recovered during the treatment phase itself. Since then, the advantage of the MDA method becomes even more apparent when long-term studies are planned. Besides, while the MDA method is easy to perform and within everyone's reach, the OG method requires specific training of the experimenter, particularly with regard to the high risk of injury (lesion of the oral cavity, pharynx, larynx, trachea, esophagus and stomach…) that would result from improper positioning of the head and body (Machholz et al., 2012).

In conclusion, this study presents a non-invasive administration method that is much closer to oral administration performed in humans and is particularly suitable for long-term administration in rats. In addition to being easy to set up, this method does not require any training for the experimenter and does not entail any risk of injury to the digestive tract. This method is particularly suitable for hydrophilic compounds and allows strict control of the time and the dose administered according to the animal's body weight.

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CRediT authorship contribution statement

Marie Heraudeau: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Candice Roux:** Conceptualization. **Caroline Lahogue:** Conceptualization. **Stacy Largillière:** Conceptualization. **Stéphane Allouche:** Resources. **Véronique Lelong-Boulouard:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Thomas Freret:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

None of the author has conflict of interest to declare.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jneumeth.2023.109951.

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