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Intranasal Administration of a Polymeric Biodegradable Film to C57BL/6 Mice

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Nasal drug delivery in rodents is a challenging procedure, especially for brain targeting, as the position of the material in the nasal cavity determines the success of the administration method. The objective of this study was to assess a novel intranasal administration technique for nose-to-brain delivery of biodegradable nasal films. The method was performed in C57BL/6 (*n* = 10; age, 8 wk) under inhaled sevoflurane. Twenty-four gauge catheters were used for the procedure. Hydroxypropyl methyl-cellulose-based film was formed in the lumen of the catheter and then delivered into the mouse nostril by pushing it out of the lumen using a trimmed and polished needle. Methylene blue was incorporated in the film-forming gel to indicate the delivery area in which the films were deposited. After administration, all mice recovered from anesthesia without incident. None of the mice showed any signs of injury, discomfort, or nose bleeding, thus allowing us to characterize the administration method as noninvasive. Furthermore, postmortem evaluation revealed olfactory-centered placement of the polymeric films, confirming the accuracy and repeatability of the method. In conclusion, this study documented the use of, a novel, noninvasive, intranasal administration technique for nose-to-brain drug delivery in biodegradable films for use in mice.

Abbreviation: NBD, nose-to-brain delivery

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Introduction

During the past decade, nasal delivery of drug substances has gained great interest as an alternative, noninvasive mode for both brain targeting and systemic administration.[7] The anatomy and physiology of the nasal cavity enable the direct transfer of drugs to the brain via the olfactory and trigeminal pathways, thus bypassing the blood–brain barrier. Many recent studies[8,20,22,23] have aimed to develop liquid or solid nasal drug formulations for the treatment of neurodegenerative diseases. Mice have already been used successfully in studies using nasal administration.[24,26] However, compared with the situation in humans, the administration of these formulations to rodents is challenging due to their specific anatomic features and markedly smaller nose.

Mouse nostrils are semicircular in shape and have a caudolateral orientation, with an inner diameter of approximately 0.42 mm.[27] This internal diameter narrows as it progresses toward the nasopharynx.[13] In rodents, the olfactory region comprises approximately 40% of the caudal portion of the nasal cavity.[3] Nose-to-brain delivery (NBD) of compounds can be achieved via the olfactory and trigeminal pathways. Administered drugs interact with the nerve endings (cilia of receptor cells) of the olfactory and trigeminal neurons in the olfactory area of the nasal cavity.[1] Consequently, drug formulations intended to target the CNS should be placed in this region. Therefore, the development of mucoadhesive formulations that adhere to the epithelial surface might increase the residence time on the nasal mucosa and thus the drug concentration in the brain.[12]

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Products currently available for NBD are either solutions or powders.[9,25,28,30,31] However, most published studies concern the nasal administration of solutions or powders reconstituted in water for injection.[15] In a recent preclinical study, a liquid formulation was administered intranasally to unanesthetized mice by using a specialized device that prevented the mouse from swallowing the administered dose.[14] This procedure did not cause any apparent reaction or discomfort in the mice, and no signs of nose irritation or breathing difficulties were reported. Nasal vaccination was accomplished by using an insufflators to administer a dry powder to anesthetized mice.[26] However, in both of these cases, the dosing and the targeting of olfactory area were inaccurate, thus decreasing both the drug levels achieved in the brain and subsequent efficacy. Some of the administered dose was absorbed into the respiratory region, and some was swallowed. Although the dose absorbed into the respiratory system might eventually reach the brain via the bloodstream, the blood-brain barrier might impede its access. Any of the swallowed intranasal dose would undergo the same metabolism as other orally administered drugs (e.g., degradation in stomach, first-pass effect, enzymatic degradation).[6] The development of intranasal dosage formulations that can placed directly in the nares to allow release of the active substance close to the neuronal bundles of olfactory sensory neurons at the lamina propria[25] will reduce both dose variability and systematic side effects.

A novel dosage form for NBD of the acetylocholinesterase inhibitor donepezil was developed recently and evaluated in vitro and ex vivo as a candidate formulation for treatment of Alzheimer disease.[21] In this formulation, hydroxypropyl methyl cellulose films were manufactured containing polyethylene glycol 400 and methyl-β-cyclodextrin, as plasticizer and permeation enhancer, respectively. The composition of the films was optimized in our previous study[21] after the application of Design of Experiments methodology. They are fast-dissolved

and biodegradable films able to be attached on the nasal mucosa and release the drug on its surface.

The aim of the current study was to develop a method to deliver this formulation intranasally to mice. The method we developed is consistent with maintaining animal welfare and does not cause the mice any harm, injury, or discomfort. Furthermore, we evaluated our administration method in terms of accuracy and repeatability by using postmortem surgical retraction of the bony roof of the nasal cavity and confirming the deposition of the nasally administered polymeric film in the targeted olfactory area.

Materials and Methods

Chemicals. Hydroxypropyl methyl cellulose (Methocel E50 premium LV; MW, 90,000 g/mol) was purchased from Colorcon (Shanghai, China). Methyl-β-cyclodextrin (MW, 1310 g/mol) was acquired from Fluka Chemika (Mexico City, Mexico). Polyethylene glycol 400 and methylene blue solution were obtained from Sigma–Aldrich (St Louis, MO).

Animals. These experiments were performed in the animal facility of the Centre of Clinical and Experimental Surgery and Translational Research (Biomedical Research Foundation, Academy of Athens). The facility is a registered breeding and experimental facility according to the Greek Presidential Decree 56/2013, which harmonizes national legislation with the European Directive 2010/63 on the Protection of Animals used for Experimental and Other Scientific Purposes.[4]

A SPF breeding colony of C57BL/6J mice was established by using mice obtained from Jackson Laboratory (Bar Harbor, ME). Male C57BL/6 mice (n = 10; age, 8 wk; weight, 23.8 ± 0.4 g) were used in the study. Mice were housed in type IIL IVC (Techniplast, Varese, Italy) under SPF and constant environmental conditions (12:12-h light:dark cycle, lights on at 0600; temperature, 22 ± 2 °C; relative humidity, 45% ± 10%). Cages each contained a Mouse House (Tecniplast) as an enrichment device. The mice were fed irradiated pellets (4RF22, Mucedola, Milano, Italy) and had access to tap water ad libitum. The cage bedding comprised corncob granules (REHOFIX, J Rettenmaier and Söhne, Rosenberg, Germany). Cages and bedding were changed once each week. All mice in the facility were screened quarterly by using a health monitoring program in accordance with FELASA recommendations and were negative for a wide range of pathogens including mouse hepatitis virus, minute virus of mice, Theiler murine encephalomyelitis virus, mouse parvovirus, murine norovirus, mouse rotavirus, lymphocytic choriomeningitis virus, mouse adenovirus 1 and 2, mousepox (ectromelia) virus, pneumonia virus of mice, reovirus type 3, Sendai virus, *Clostridium piliforme*, *Mycoplasma pulmonis*, β-hemolytic *Streptococcus* spp., *Streptococcus pneumonia*, *Bordetella bronchiseptica*, *Pasteurella* spp., *Pasteurella pneumotropica*, *Helicobacter* spp., *Corynebacterium kutscheri*, *Citrobacter rodentium*, *Salmonella* spp., *Streptobacillus moniliformis*, *Cryptosporidium* spp., fur mites, and pinworms.[10] The experimental protocol of the study was approved by the Veterinary Authorities of Region of Athens, Greece (reference no. 30643/15-04-2021).

Preparation of the polymeric films. Drug–free polymer gel was prepared to contain 1.5% (w/w) hydroxypropyl methyl cellulose E50, 1.7% (w/w) polyethylene glycol 400, and 0.8% (w/w) methyl-β-cyclodextrin in sterilized water. Briefly, hydroxypropyl methyl cellulose was dispersed in hot water (above 80 °C), and then hydrated at lower temperature (below 10 °C) for 15 min.[5] Before hydration, polyethylene glycol 400 and methyl-β-cyclodextrin were added under continuous magnetic stirring. To allow us to visualize where the film was deposited in the nasal cavity, sufficient methylene blue was suspended in the gel to achieve a uniform blue color. The film was formed in the lumen of a 24-gauge catheter from which the needle had been removed. Aspiration of a weighed amount of gel (2.5 ± 0.3 mg; Figure 1 A) and drying at room temperature (25 °C) for 72 h led to the formation of a cylindrical film (0.10 ± 0.03 mg) in the lumen of the catheter (Figure 1 B); the

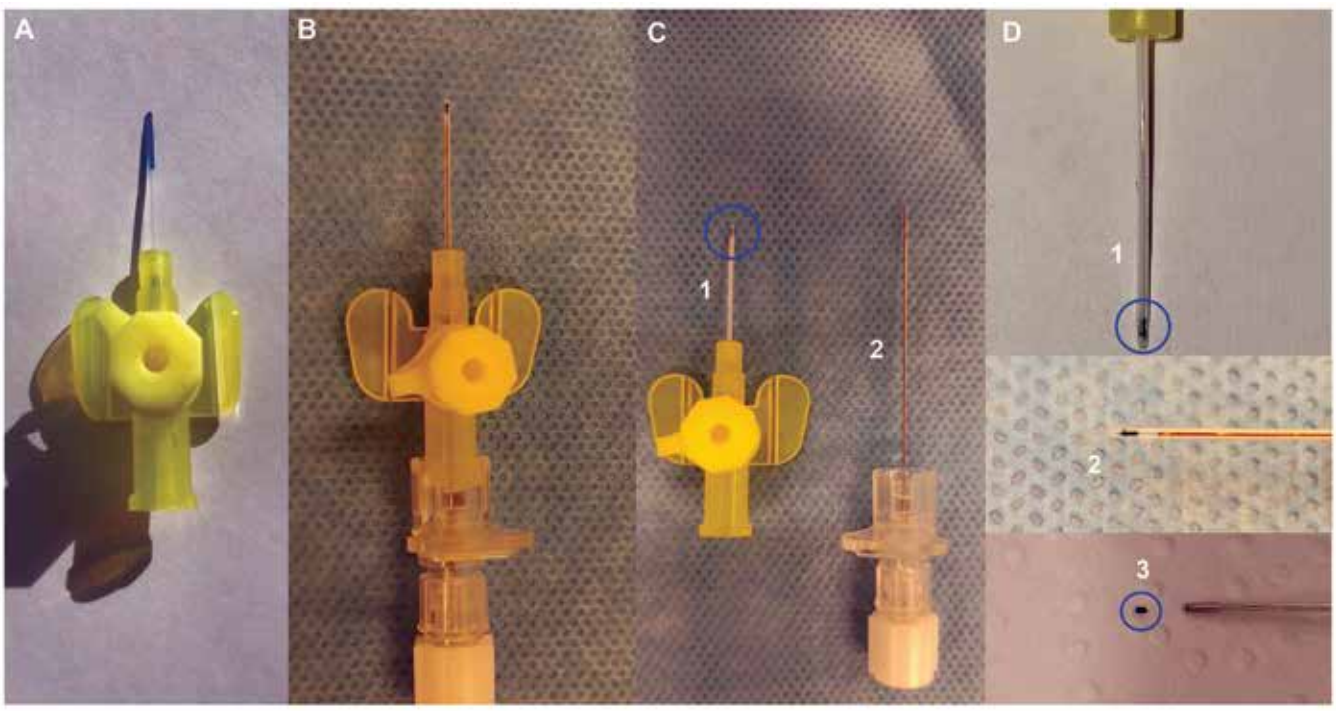

Figure 1. A) Aspiration of weighed amount of film-forming gel. B) Device for the nasal delivery of blue polymeric film in mice. C) The intranasal delivery device consisted of a 24-gauge catheter (1) and a cut and polished needle (2). D) The film is formed in the lumen of the catheter (1) and can be dislodged by using the needle (2 and 3).

film was dislodged from the lumen by using the needle (Figure 1 C and D). To minimize the risk of harm or injury during administration, the tip of the needle was cut to be the same length as the catheter tube and then polished.

Anesthesia. For intranasal administration of the film, mice were first anesthetized by using sevoflurane (Sevorane, Abbott France, Saint Remy sur Avre, France) via a calibrated vaporizer (Abbott Laboratories, IL) at a minimum alveolar concentration of 2% to 4% in 100% oxygen. The anesthetic agent was delivered through an inhouse-3D–printed rodent face mask with a coaxial design to secure the supply and scavenging of the gas (Figure 2). The 3D-printed mask allows administration of gas anesthesia without escape into the room. Mice were monitored for any signs of abnormal breathing during the anesthesia, and a negative foot reflex was used to indicate a surgical plane of anesthesia.[19]

Nasal administration of polymeric films. A flexible, nontraumatic plastic catheter (diameter, less than 0.7 mm) was used to guide the film into a nostril. The investigator used one hand to gently restrain a mouse via the loose skin of the neck; mice then were placed with their heads down and turned to the side to facilitate installation of the catheter into a nostril. Before insertion, the needle was placed on but not locked onto the catheter. The tube was inserted approximately 0.5 cm into the right nostril at an angle of 20° to 25° and then gently aligned in the direction of the nostril (Figure 3 A). At the 0.5-cm point, resistance was encountered, and the catheter could not be advanced any further. Subsequently, the film was introduced in the right nostril of the mice by locking the needle onto the catheter (Figure 3 B).

Figure 2. C57BL/6 mouse receiving sevoflurane anesthesia delivered via a 3D-printed face mask.

Surgical retraction of the bony roof of the nasal cavity. After nasal administration of the film, mice were allowed 3 min for recovery from the procedure. We then randomly selected and euthanized 5 mice and surgically retracted the bony roof of their right nostril, to evaluate the position of the film in the nasal cavity. The remaining 5 mice were observed for 24 h to identify any abnormal behavior, including abnormal breathing, attempts to scratch the nose and remove the film, and nose bleeding. The 5 mice were regularly monitored during the 24 h after administration to ensure that they expressed typical behaviors (e.g., normal movement, social and self-grooming, chasing, standing, tail wagging, drinking water, and eating).

Mice were euthanized by cervical dislocation under anesthesia with high-dose sevoflurane (minimum alveolar concentration, 7% to 8% in 100% oxygen). Mice were then decapitated, and a longitudinal section of the skin, starting at the base of the skull, was removed by using microsurgery scissors. The skin was detached from the cranial bone at the front part of the nose. An 8-mm, longitudinal section of the right nostril revealed the right side of the nasal cavity, allowing the position of the film to be evaluated.

Results

We used 24-gauge catheters to intranasally administer a hydroxypropyl methyl cellulose–based film to C57BL/6 mice; the lumen of the catheter was used as mold to form the cylindrical films. The resulting films (n =10; length, 0.90 ± 0.12 mm; diameter, less than 0.7 mm) were blue, atraumatic, and biodegradable. The administration procedure required 3 min for anesthesia and less than 1 min to place the film. We confirmed correct placement of the film into the nasal cavity nonsurgically and in some cases by surgery and visualization. The catheter was weighed before and after placement in order to indicate absence of the cylindrical film from the lumen of the catheter. Successful application was achieved in all 10 mice. None of the mice showed bleeding from the nose after administration, and all mice recovered successfully from anesthesia.

Once the mice had recovered completely from anesthesia, we randomly selected 5 of them and surgically retracted the bony roof of the nasal cavity. We then opened the right nostril to reveal the blue-stained mucosal tissue (Figure 4, blue arrow), indicating the position of the film in the olfactory region. The blue-stained area (approximately 0.35 mm^2) could be seen either on the top or at the bottom of the nostril approximately 8 to 9 mm from the nostril outlet, suggesting that the film was attached to the surface of nasal mucosa, without any sign of drainage toward the nasopharynx. This successful film positioning was confirmed in all 5 mice that were evaluated through surgical retraction of the bony roof of the right nostril.

We consider that the mice experienced minimal physical stress because the procedure was performed under anesthesia and because we detected no nose injury in any of them. Furthermore, the 24-h observation period after intranasal administration of

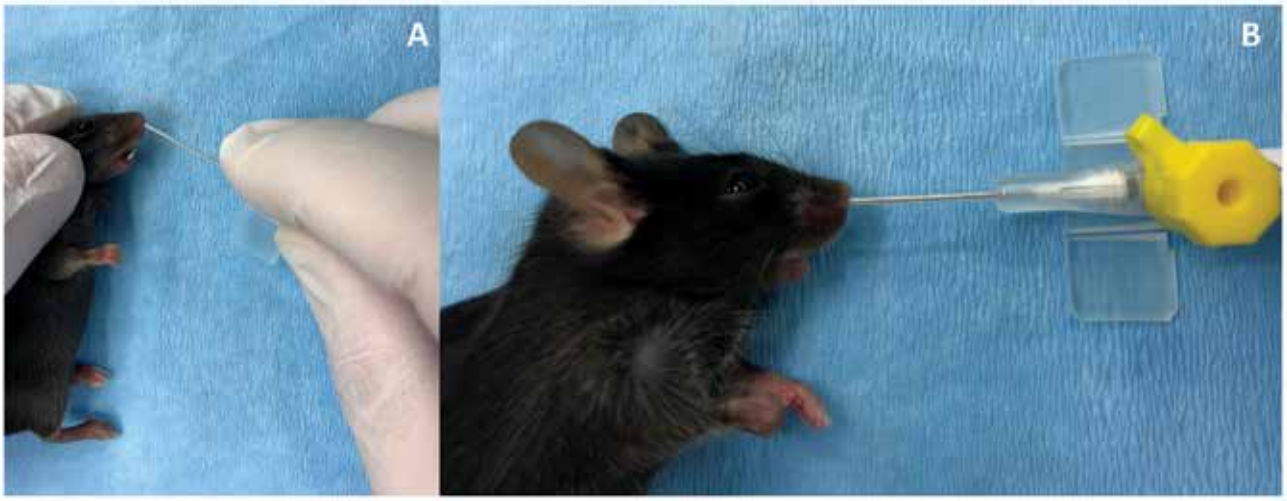

Figure 3. Immobilization of a mouse and appropriate positioning of its head for intranasal administration of the polymeric film. A) Before and B) after introduction of the catheter into the nostril.

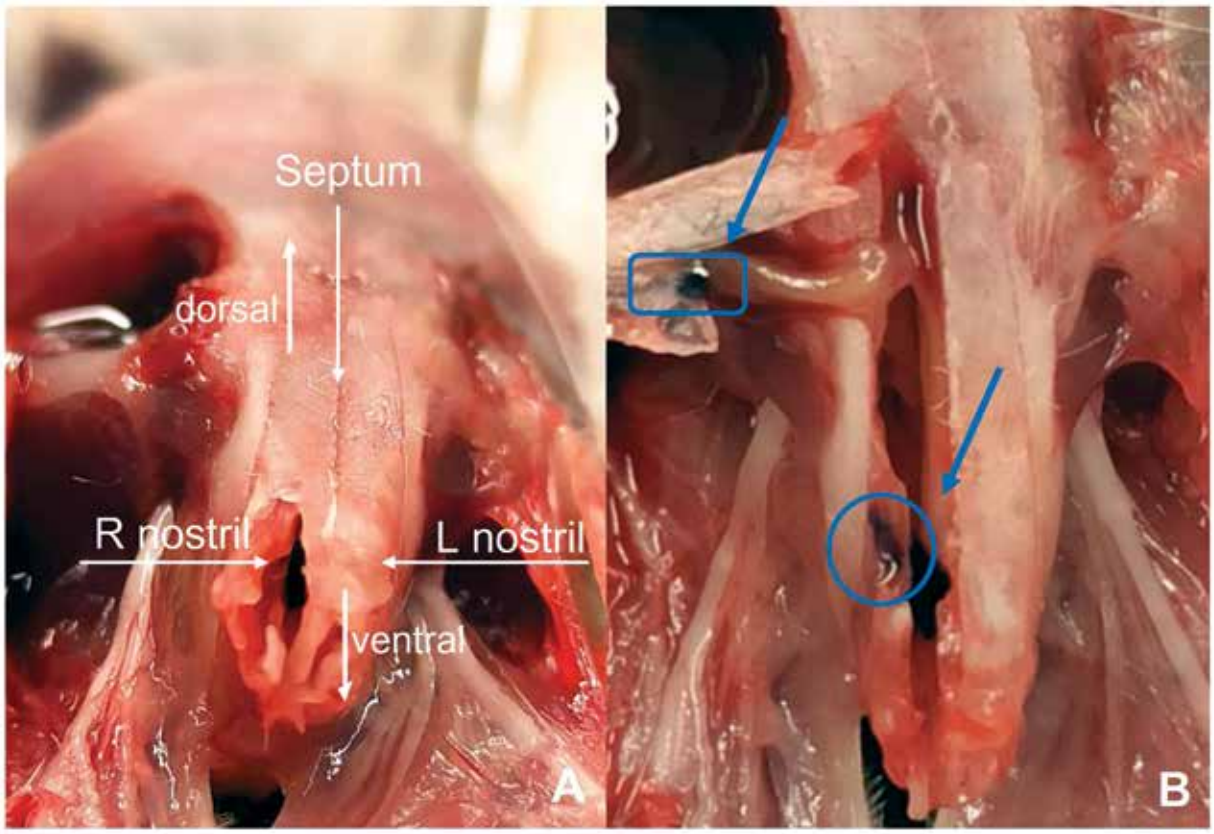

Figure 4. Mouse nose bone A) before and B) after retraction of the bony roof. The blue arrow indicates the placement of the polymeric film in the right nostril.

the polymeric film did not reveal any abnormal behavior in any of the mice.

Discussion

Nasal administration in mice is a challenging procedure because it requires highly controlled manipulation of the animal due to the small dimensions of their nostrils[8] and the tendency of mice to remove foreign objects from the nasal cavity. The methods currently available primarily involve the administration of powders or liquid solutions, with or without anesthesia.[14-16,26] However, liquid formulations present several disadvantages that reduce its effectiveness as a NBD dosage form. Dose variability and rapid nasal drainage can both occur when a solution is administered intranasally, whereas more viscous formulations such as gels can be applied more uniformly. In this context, the development of gel formulations that can be dried to form flexible films may offer an alternative approach for accurately delivering drug doses to the olfactory area. In the current study, we used a trimmed and polished needle as a piston to

dislodge the film from the catheter lumen and deliver it inside the nasal cavity. We opted not to use syringe air as a mode of administration, because that method would not allow controlled region-specific delivery of the formulation in the nostril.

In the literature, the most common method used for intranasal administration involves nasal solutions and can be achieved by using an appropriate restraint method, which allows administration without anesthesia.[14] This method can be used in mice with severe disease phenotypes, even if daily dosing is required, without the need for accompanying anesthesia. To prevent drainage of the administered drug into the lungs or stomach, one group developed a positioning device that ensured the correct placement of mice for intranasal administration.[32] Although this approach improved the effectiveness of the method, dosage inconsistency due to the drug formulation remained.[2] Intranasal administration can also be performed during inhalation anesthesia or through reverse cannulation via the esophagus by using a syringe pump. However, intranasal administration during inhalational anesthesia leads to dosage inaccuracy, and reverse cannulation is time-consuming, invasive, and is a terminal procedure.[16]

Nasal powders have also been used for intranasal drug delivery and are more stable and resistant to mucociliary clearance than solutions.[17] However, only a few preclinical studies on intranasal powders for brain targeting in mice have been published. One group[26] aimed to achieve nasal vaccination of mice by using an insufflator to administer micro- and nanoparticles. Because the insufflator was introduced into the nostril, the dose was administered diffusely throughout the nasal cavity and not in a region-specific manner.

Catheters can easily be used for nasal delivery because no specialized equipment is required. For example, one group[11] used a catheter in an olfactory region-specific intranasal technique for NBD of liquid formulations to neonates. This method is promising with regard to reducing dose variability problems in pharmacokinetic and pharmacodynamic studies of nasal solutions. The technique we propose in the current study aims to establish an administration method for the new dosage formulation of nasal films in mice. We expect that this dosage formulation will gain substantial acceptance in the field of nasal delivery. Recently, 2 studies reported using nasal films for topical treatment of dry nasal syndrome and early-stage Covid-19.[18,29] Our previous work[21] is the first published report regarding the development of nasal films for brain targeting; the current study aims to document consistent NBD of drugs to the olfactory area of mice. We are currently measuring donepezil levels in brain tissues and serum after intranasal administration to C57BL/6 mice to determine whether our administration method can deliver appropriate doses of donepezil-containing nasal films to the CNS and bloodstream.

To conclude, the results of the present study document the effectiveness and repeatability of the described intranasal administration method for mice. The method is considered a procedural refinement, because the mice that participated in the study did not experience any "harm, pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle," as stipulated in Directive 2010/63/EU. The short duration of the administration procedure and the fact that the method requires no specialized equipment render it appropriate for the delivery of solid formulations, in film form, to the olfactory region of the mouse nostril. Surgical retraction of the bony roof of the nasal cavity showed that the adhesive properties of the formulation impede the drainage toward the nasopharynx, thus promoting greater accuracy of the administered dose. The in vivo performance of drug-containing nasal films will reinforce the applicability of this administration method for NBD applications. Regardless of its ultimate utility for NBD, the proposed intranasal technique is appropriate for topical or systemic delivery, ensuring dose consistency and minimal animal pain and distress.

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