Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences
Tome 78, No 10, 2025

BIOLOGY
Neurobiology

NOSE-TO-BRAIN DELIVERY OF INDOCYANINE GREEN IN RATS

Milena Mishonova[1], Lea Koceva[1], Bissera Pilicheva[2], Plamen Zagorchev[2], Petar Eftimov[3], Hristo Gagov[1], Iliyana Sazdova[1]✉

Received on July 20, 2025
Presented by N. Atanassova, Corresponding Member of BAS, on September 30, 2025

Abstract

Nose-to-brain drug delivery is a promising method for the direct introduction of chemicals and structures into the brain, bypassing the systemic circulation and the blood-brain barrier. Indocyanine green (ICG) is a near-infrared-emitting dye that is minimally toxic to humans. It is widely used in photodynamic applications, particularly in angiography and for many intraoperative staining procedures. In our study, ICG was administered intranasally to rats, and its penetration into the brain was observed. ICG distribution was measured using a near-infrared excitation laser at 1, 4, and 24 hours after administration as a solution into the rat nasal cavity near the olfactory mucosa. As a result, significant staining of the olfactory bulb and brainstem – mainly in the basolateral region of the pons – was observed in all cases, with the strongest signal detected at four hours. A more rapid decline in signal intensity was observed in the brainstem compared to the olfactory bulbs. These findings suggest that ICG readily crosses the nasal mucosal barrier and reaches the olfactory bulbs and brainstem via the olfactory and trigeminal nerve pathways, respectively. Additionally, ICG appears to be a useful dye for labelling molecules and supramolecular structures in studies of nose-to-brain delivery and whole-body distribution.

Key words: near-infrared, olfactory bulb, olfactory mucosa, trigeminal nerve, transcellular, paracellular

This research was funded by the Ministry of Education and Science of Bulgaria, Grant DO1-361/2023 "INFRAACT" of NRRI 2020–2027.

`https://doi.org/10.7546/CRABS.2025.10.04`

Introduction. Indocyanine green (ICG) is a fluorescent, tricarbocyanine water-soluble dye that is minimally toxic to humans [1]. ICG is a suitable contrast agent for in vivo measurements due to its near-infrared (NIR) emission wavelength (800–830 nm) that allows for greater penetration depth and emission in tissues and organs, as it does not interfere with the autofluorescence of cells and blood, which express strong emission intensity in the 500–600 nm range [1–3].

The nose-to-brain pathway is a non- or minimally invasive route of administration into the central nervous system (CNS) that is often much more effective than oral or intravenous application [4, 5]. Small molecules up to 1000 Da, when applied to the surface of the nasal mucosa, could readily overcome this barrier for translocation to different brain regions by trans- or paracellular transport, using the trigeminal nerve pathway towards the brainstem (the basolateral region of the pons) and the olfactory neurons (olfactory pathway) to the olfactory bulb and the cerebrospinal fluid [5, 6]. Large molecules, such as insulin, basic fibroblast growth factor, monoclonal antibodies, oligonucleotides, as well as some drug-loaded nanoparticles and nanostructures, can also reach different regions of the brain and cerebrospinal fluid, thereby bypassing the systemic circulation and the blood-brain barrier through intranasal administration [5, 6].

The present study aimed to investigate the penetration of externally applied ICG through the nasal mucosa for its nose-to-brain delivery.

Materials and methods. All experimental procedures were conducted following the Guiding Principles for Care and Use of Laboratory Animals approved by the Bulgarian Center for Bioethics, the Ethics Committee for Animal Experiments of Sofia University, and complied with the International Guiding Principles for Biomedical Research Involving Animals, as issued by the Council for International Organizations of Medical Sciences. We have ethical approval from the Bulgarian Food Safety Agency, Ministry of Agriculture, Food and Forestry (No. 224, 23 January 2019) and from the local Ethics Committee of the Faculty of Biology, Sofia University "St. Kliment Ohridski" (Protocol No. 2/2022).

ICG solution and its administration into the nasal cavity. ICG dissolved in DMSO was diluted with sterile water for injection to a final concentration of 160 µg/mL. Wistar rats of age 3 months and weighing 329.11 ± 22.5 g ($n = 18$) were used. The animals were anesthetized with an inhalation anaesthesia, a mixture of isoflurane and oxygen (4% isoflurane for induction, followed by 1.5% for maintenance for 5 min), then injected with Nalgosed® (2 mg/kg) and Sedin® (3.75 mg/kg) before intranasal administration of ICG or decapitation. ICG was applied in the upper 1/3 of the nasal cavity via a venous cannula (G-23), 40 µL in each nostril.

The animals were divided into three groups: the brain was isolated and imaged 1 h, 4 h, or 24 h after ICG application, respectively. The anesthetized animals of each group were decapitated with a guillotine. The temporal and nasal bones were removed, and the brain was gently excised. It was placed in a Petri dish, and an image was acquired in the Imaging system at wavelengths of 700 and 800 nm in the dorsal and ventral planes.

Infrared fluorescence (IRF). IRF was measured using the Pearl Trilogy Small Animal Imaging System (LI-COR Biotechnology GmbH, Bad Homburg, Germany). ICG (infrared emission dye) has a typical Stokes luminescence with an excitation range of 750–800 nm and an emission fluorescence peak at 830 nm [7]. In the present study, ICG fluorescence was activated by a 785 nm laser, and the emission was measured at 800 nm. The penetration and distribution of ICG in brain structures were assessed by the intensity of fluorescence compared to the background signal. The signal is presented as the difference between the background and the fluorescent area.

Statistical analysis. Statistical analysis was performed using the R statistical programming software, version 4.4.0 (Copyright © 2024, The R Foundation for Statistical Computing). Data are presented as mean ± standard error of the mean (SEM). Differences between groups were assessed using Student's t-test for independent samples. A value of $p < 0.05$ was considered significant.

Results and discussion. Representative images of the fluorescence emission are shown in Fig. 1. The signal is stronger from the olfactory bulbs compared to

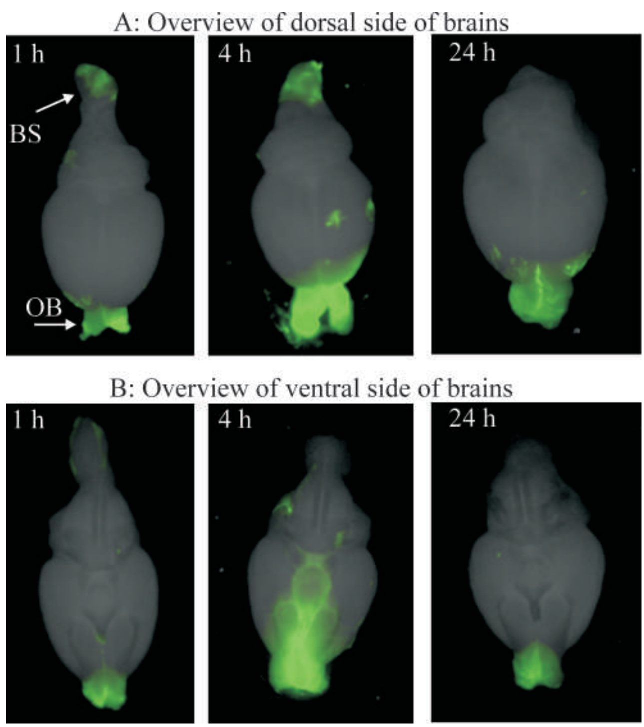

Fig. 1. Representative images of ICG staining of the rat brain. Emission from the dorsal side (A) and the ventral side (B) of the rat brain excised 1, 4, and 24 h after intranasal administration of ICG. Arrows indicate olfactory bulbs (OB) and brainstem (BS)

the brainstem area, which is statistically significant on the 24 hour ($p = 0.0296$,*). A slight difference in fluorescence was observed between ventral and dorsal views, especially at the first hour, but it was not significant ($p = 0.078$, ns).

One hour after ICG administration, a signal was detected in the olfactory bulbs and the brainstem with similar intensity (Fig. 2). The most intense fluorescent signal was measured four hours after application, when ICG had spread to different areas of the brain from the site of primary penetration (olfactory bulbs and brainstem). Twenty-four hours after intranasal administration, the signal in the brainstem had almost disappeared, but the olfactory bulbs still emitted a weak signal.

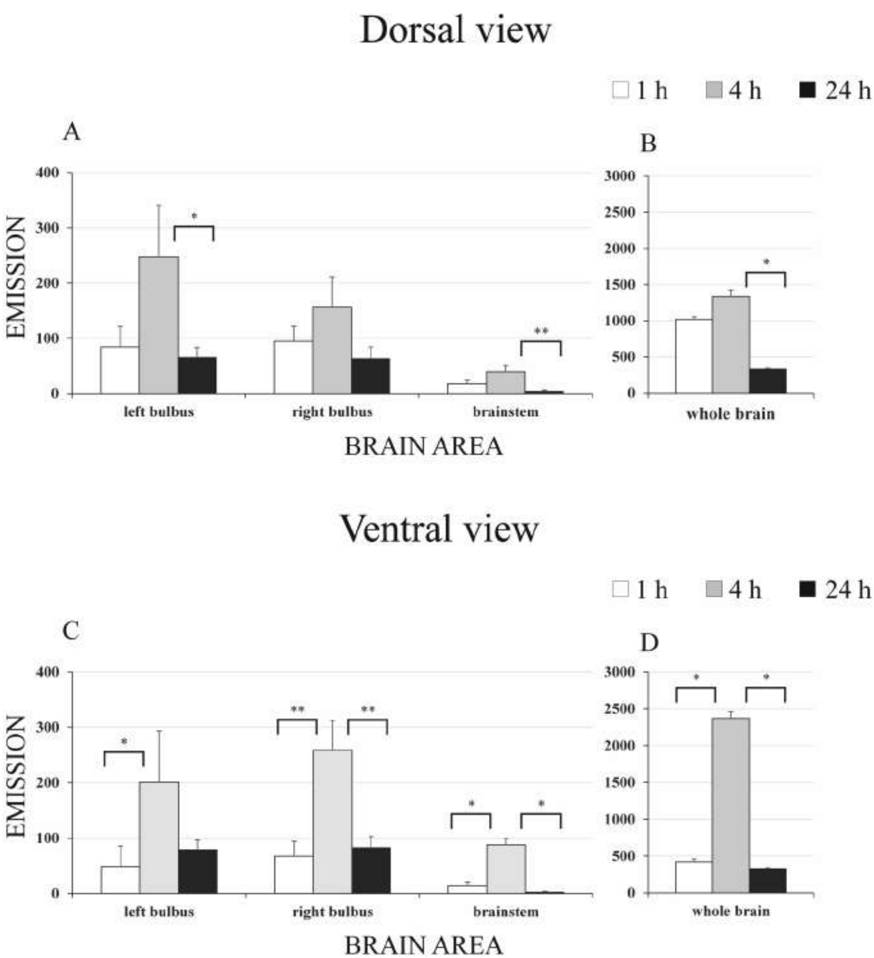

Fig. 2. Average fluorescence intensity from individual brain sections from the dorsal (A) and the ventral (C) surface. Overall fluorescence intensity from the entire brain: B – dorsal view; D – ventral view. The strongest emission was measured 4 h after ICG administration (■) compared to 1 (□) and 24 (■) h. Data are presented as mean ± standard error ($n = 6$). $^{**}p < 0.01$; $^{*}p < 0.05$

Until recently, there was no data on rat brain staining after intranasal administration of ICG. In March 2025, a paper was published in which ICG suspensions were administered both orally and intranasally, and brain staining in rats was recorded using semi-quantitative in vivo analysis of ICG emission in the near-infrared region at a wavelength of 840 nm [8]. This method saves the rats alive but in the case of intranasal administration it has some disadvantages compared to recordings from excised brains, namely: i) the signal is mixed from the brain and intranasal regions, making semi-quantitative analyses incorrect; ii) it is used only for dorsal measurements, and iii) it does not allow the comparison of two main pathways for nose-to-brain delivery, which use the olfactory and trigeminal nerves via transcellular and/or paracellular mechanisms. For these reasons, we consider our measurements to be more relevant, as they monitor the involvement of both possible delivery pathways from the nasal cavity to the brain (Fig. 3). They allow for comparison of delivery capacity and the direction of the substance into different brain regions. Our methodology for inputting and imaging the fluorescent signal from the administered ICG allows visualization in both planes (dorsal and ventral). As shown in Fig. 1, the ventral signal is stronger compared to the dorsal view. The images acquired from the excised brain are free of disturbing background signal from the nasal cavity. In addition, these images are more detailed when compared to previously reported recordings in native skulls, due to the limited penetration depth through the cranial bones.

It is well known that ICG is widely used in the angiography of choroidal and retinal circulation [9], as well as for precise visualization of hard-to-access tumours in neurosurgery [10], facilitating their resection as safely as possible. In these cases, ICG is typically administered intravenously the day before the procedure; however, alternative routes of administration are poorly studied. Our results suggest that intranasal (nose-to-brain) administration of ICG can reduce both the time and the total amount of dye required for neurological examinations, thereby minimizing systemic exposure.

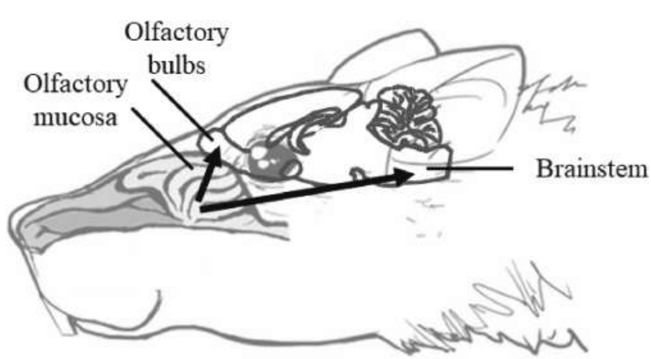

Fig. 3. Scheme of possible transport pathways after nasal administration. Black arrows indicate the area of distribution in the brain

Furthermore, when incorporated into supramolecular structures such as nanoparticles, liposomes, solid lipid nanoparticles, and dendrimers, ICG allows researchers to visualize the movement of such carriers from the nasal cavity to the brain, optimize delivery strategies, and evaluate the effectiveness of different formulations. Such imaging could be beneficial for studies on drug delivery from the nose to the brain for revealing how nanosized carriers are transported along neural pathways [11]. It can help optimize drug formulation by visualizing the effects of particle size, chemical composition, or chemical modifications on drug distribution [12]. It can also be used to quantify the carrier load reaching the brain and guide the selection of appropriate delivery regimens by revealing how the signal/concentration changes over time in the nasal passages and the brain, and for how long the carrier remains there, which is essential for achieving a sustained therapeutic effect.

Conclusion. Our data demonstrate that ICG readily crosses the nasal mucosal barrier. This is the first evidence that ICG utilizes both the olfactory and trigeminal pathways to rapidly reach the olfactory bulbs and brainstem regions. ICG appears to be a useful dye for labelling molecules and supramolecular structures, allowing simultaneous monitoring of their nose-to-brain delivery.

REFERENCES

[1] Lim Z. Y., S. Mohan, S. Balasubramaniam et al. (2023) Indocyanine green dye and its application in gastrointestinal surgery: The future is bright green, World J. Gastrointest. Surg., **15**(9), 1841–1857, `https://doi.org/10.4240/wjgs.v15.i9.1841`.

[2] Marasini R., S. Aryal (2022) Indocyanine-type infrared-820 encapsulated polymeric nanoparticle-assisted photothermal therapy of cancer, ACS Omega, **7**(14), 12056–12065, `https://doi.org/10.1021/acsomega.2c00306`.

[3] Alius C., S. Oprescu, C. Balalau, A. Elena Nica (2018) Indocyanine green enhanced surgery; principle, clinical applications and future research directions, J. Clin. Invest. Surg., **3**(1), 1–8, `https://doi.org/10.25083/2559.5555/31.18`.

[4] Crowe T. P., W. H. Hsu (2022) Evaluation of recent intranasal drug delivery systems to the central nervous system, Pharmaceutics, **14**(3), 629.

[5] Patharapankal E. J., A. L. Ajiboye, C. Mattern, V. Trivedi (2023) Nose-to-brain (N2B) delivery: An alternative route for the delivery of biologics in the management and treatment of central nervous system disorders, Pharmaceutics, **16**(1), 66, `https://doi.org/10.3390/pharmaceutics16010066`.

[6] Keremidarska-Markova M., I. Sazdova, M. Mladenov et al. (2024) Sirtuin 1 and hormonal regulations in aging, Appl. Sci., **14**(24), 12051, `https://doi.org/10.3390/app142412051`.

[7] Miwa M. (2010) The principle of ICG fluorescence method, Open Surg. Oncol. J., **2**, 26–28.

[8] Samala S., A. Mhaske, R. Shukla (2025) Intranasal Apocynin-loaded nanostructured lipid carriers (NLCs) for Alzheimer's disease therapy: Formulation, op-

timization, and pharmacokinetic evaluations, J. Drug Delivery Sci. Technol., **108**, 106862, https://doi.org/10.1016/j.jddst.2025.106862.

[9] Delgado-Tirado S., L. Gonzalez-Buendia, L. A. Kim (2021) Indocyanine Green Angiography. In: D. M. Albert, J. W. Miller, D. T. Azar, L. H. Young (Eds), Albert and Jakobiec's Principles and Practice of Ophthalmology, 2683–2712, Cham, Springer, https://doi.org/10.1007/978-3-030-42634-7_139.

[10] Cho S. S., R. Salinas, J. Y. K. Lee (2019) Indocyanine-green for fluorescence-guided surgery of brain tumors: evidence, techniques, and practical experience, Front. Surg., **6**, 11.

[11] Veronesi M. C., M. Alhamami, S. B. Miedema et al. (2020) Imaging of intranasal drug delivery to the brain, Am. J. Nucl. Med. Mol. Imaging, **10**(1), 1–31.

[12] Almahmoud A., H. S. Parekh, B. M. Paterson et al. (2024) Intranasal delivery of imaging agents to the brain, Theranostics, **14**(13), 5022–5101, https://doi.org/10.7150/thno.98473.

[1]*Department of Animal and Human Physiology, Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tsankov Blvd, 1164 Sofia, Bulgaria*
e-mails: mmishonova@biofac.uni-sofia.bg, kocevalea@gmail.com, hgagov@uni-sofia.bg, i.sazdova@biofac.uni-sofia.bg

[2]*Faculty of Pharmacy, Medical University of Pleven, 1 St. Kliment Ohridski St, 5800 Pleven, Bulgaria*
e-mails: bisera.pilicheva@mu-pleven.bg, plamen.zagorchev@mu-pleven.bg

[3]*Department of Cell Biology and Development Biology, Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tsankov Blvd, 1164 Sofia, Bulgaria*
e-mail: peftimov@uni-sofia.bg