Technical note

Technique for administering drugs to the brain and bypassing the blood-brain-barrier

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Introduction
Drugs do not readily pass from the bloodstream and into the substance of the brain. This is the result of a barrier called the blood-brain-barrier, which is made up by glial cells, surrounding the normal capillary endothelial cells in the brain. These glial cells function as a metabolic and physical barrier to substances and selects which chemicals may enter into the brain. That is why the delivery of drugs into the brain is severely impeded and the transfer across this barrier is restricted to non-polar, hydrophobic molecules. Due to this problem many drugs, which do not cross this barrier, need to be injected directly into the subarachnoid space by lumbar puncture or cisternal puncture (Allison & Stack 1978).

Numerous works have shown that if dyes are injected into the subarachnoid space of the brain, these substances pass into the nasal mucous membrane, in low concentrations, and also into the lymphatic system of the neck (Yoffey 1949, Seki 1964). It has been shown that 32P, 198Au and Cd(II) (Czerwińska 1970, Ewans & Hastings 1992) may be found in the cerebrospinal fluid after submucosal injection in the olfactory region. These results indicate that drugs may be transported across the olfactory membrane directly into the brain, bypassing the blood-brain-barrier. In order to confirm this theory, a technique for the mouse and rat has been developed, where drugs are sprayed or inserted directly on regio olfactoria, whereafter samples may be collected in order to measure the content of delivered drugs in different regions of the brain or the drug concentration in the cerebrospinal fluid.

Materials and methods
Animals
Healthy BALB/c mice (15–25 g) and inbred Wistar rats (= 250 g), both male and female, were obtained from Keldur (Institute for Experimental Pathology, University of Iceland) and used in all experiments. The animals were delivered from Keldur at the same day as the experiments were conducted. The animals had not been used in other experiments prior the study.

Procedures
The animals were anaesthetized using intraperitoneal injection of 10 mg/ml aqueous pentobarbital (Mice: 74 mg/kg; rats: 68 mg/kg) (Lyjávæslun Islands hf, Reykjavík, Iceland) solution with 15% propylene glycol (Norsk Medisinaldepot, Oslo, Norway).

Some animals received ether anaesthesia (Merck Schuchardt, Darmstadt, Germany) prior to i.p. pentobarbital injection. The animals were fixed in an appropriate restrainer, prior to the experiment.

The device for the olfactory drug delivery was a modified pipette tip. The tip of a G24×1″ needle (about 1.5–2 cm long) was rasped off from the pointed end with a grinding wheel. The point was blunted and the rear end fixed into the narrow front end of a < 200 μl pipette tip (Fig. 1) using cyanoacrylate ester glue. The tip was connected to a micropipette, ready for use.
The intraolfactory drug delivery was carried out by fixing the animal in supine position or on one side, the labia were gently pushed aside and the upper and lower incisor teeth forced apart in order to gain access to the palate. The needle was carefully inserted a few mm into the nasal cavity by penetration of the incisive duct at the palate as shown in Fig. 2. This procedure was made with care, since the needle may damage the conchae and cause major bleeding. Now, 25 µl of the drug (Gizurarson 1990) may be administered directly into the olfactory area (Fig. 3). This procedure was necessary in small animals, since the traditional intranasal installation of drugs did not deliver the drug into the olfactory area, but more to the respiratory area (Gizurarson 1990).

Collection of serum samples
For a pharmacokinetic analysis, blood samples are needed. They may be collected after appropriate time, depending on the study, according to standard methods and stored at -18°C.

Collection of brain samples from the mouse
After an appropriate time the animal is decapitated. The cranium is cleaned for skin and tissue, whereafter a cut is made in the frontal bone, between the orbitals. The cranium is now carefully cut in half along the median line. The brain is carefully removed and sectioned in such way that it is possible to measure the drug concentration in different regions of the brain. Normally, six slices
25 μl of the drug is administered in small amounts to the animal by intranasal instillation into the nasal cavity (Fig. 3). The drug is then removed from the nasal cavity and collected for analysis. Blood samples can be collected according to approved methods.

The animal is decapitated and the brain is removed and placed in a chilled solution (-22°C) to preserve the tissue. Slices of the brain are then made, and the olfactory bulbs and tractus olfactorius are separated from the first slice. Each slice is placed into a sample tube and stored at -22°C.

Collection of CSF samples

Sampling of cerebrospinal fluid was carried out by a cisterna magna puncture. An anesthetized rat is placed sideways with the head fixed forward, the neck of the rat is shaved, a small cut is made in the skin and the occipital bone is followed down to the atlantooccipital space where the puncture can be made. Using a 27G x 0.5” needle, the cerebrospinal fluid is carefully withdrawn from the cisterna magna. Sample volumes up to 150 μl may easily be collected (Fig. 4).

Results and discussion

In experiments, using diazepam as a model drug, this technique has shown that high concentrations of diazepam appear in various regions of the brain, as quickly as 2 min after the intraolfactory delivery. Similarly, when diazepam is administered intravenously it may be seen shortly after the administration, but mainly in other regions of the brain, than seen after intraolfactory administration (Gizararson et al. 1995). A simple experiment using brilliant blue (Merck Schuchard, Darmstadt, Germany) dissolved in glycerol (Roche, Basle, Switzerland) was used to evaluate the exposed area after intranasal instillation and after intraolfactory delivery of this solution. Administration through the incisive duct caused staining of the entire nasal cavity of the mouse.

![Diagram](image-url)

**Figure 3.** Paramedian section through the head of the rat, showing how the intraolfactory administration is carried out (lo). 1: Vestibulum nasi, 2: Dorsal nasal conchae, 3: Middle nasal conchae, 4: Ventral nasal conchae, 5: Pharynx, 6: Incisor teeth, 7: Ethmoidal conchae, 8: Bulbus olfactorius.

![Diagram](image-url)

**Figure 4.** Cisterna magna puncture for the collection of cerebrospinal fluid samples from rats.
especially the olfactory region, whereas the intranasal instillation of the same solution only covered the anterior (non-olfactory) region of the nasal cavity. These results show that it may be possible to administer drugs through the incisive duct to the olfactory region of the nose of laboratory animals. Further experiments are required to answer the questions if this route may be clinically relevant with respect to type of drugs, concentration, ease of delivery, frequency of dosing etc.

Acknowledgements
This work was supported by the Icelandic Student Innovation Fund and by the National Research Council of Iceland.

Summary
Drug delivery to the olfactory region may result in absorption to the brain without entering the bloodstream, thus bypassing the blood-brain barrier. A simple technique is described for administering drugs to the olfactory region in mice and rats. During anaesthesia with i.p. pentobarbital administration, the drug is administered directly to the olfactory region in the nasal cavity, through the incisive duct, using a modified needle-pipette tip device. After the drug delivery, samples may be collected from the cerebrospinal fluid or from various regions of the brain.

Résumé
Hvis et lægemiddel er placeret på lugteområdet, vil det resultere i en absorption til hjernen, forbi blod-hjerne-bARRIEREN. Artiklen beskriver en simpel teknik for at indgive lægemiddel på lugteområdet i mus og rotter. Under anæstesi med i.p. pentobarbitalindgift, er lægemiddlet givet direkte på lugteområdet i næsekaviteten, via mund-næse hule (incisive duct), ved hjælp af en modificeret nåle-pipettestift. Efter lægemiddelindgiften kan spinalvæske og prøver fra hjernens forskellige dele udtages fra dyret.

References