

General anaesthesia in rats undergoing experiments on the central nervous system

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The rat is one of the species most commonly used in laboratory practice. Numerous publications concerning various aspects of morphology and physiology are based on the results obtained in this species. It makes these results comparable and under some precautions enables to transpose into the relationships observed in humans. Each experimental project must obtain the permission of the Local Ethical Committee, as well as comply with the regulations of the European Communities Council, outlined in the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes". Adequate pre-operative care can eliminate or reduce the incidence of many complications, which may occur during anaesthesia. General anaesthesia in experimental practice can be achieved using a variety of drugs and ways of administration, among others inhalational or intravenous. The side effects of anaesthetic agents can be reduced in this way. Knowledge of the effect of anaesthetics on the cerebral circulation, metabolism and intracranial pressure in both normal and pathological conditions is crucial for neurobiological purposes. Many anaesthetic agents depress respiration, which can result in hypoxia, hypercapnia and acidosis. To maintain blood carbon dioxide and oxygen concentration in the physiological range, it is necessary to apply tracheal intubation and artificial ventilation. However, even when using sophisticated equipment, the role of basic clinical observation, such as the colour of the blood shed in the operation field, breathing depth and frequency, cannot be overestimated. The importance of monitoring mean arterial blood pressure and intracranial pressure in experiments on the central nervous system is fundamental. Special attention should be paid to controlling the temperature and monitoring the fluid balance. Appropriate postoperative care can have a decisive influence on the final results of the research.

key words: rat, general anaesthesia, intubation, artificial ventilation, monitoring

INTRODUCTION

Rats represent one of the most commonly used species in laboratory practice. Their behavioural and alimentary requirements are moderate and can be fulfilled in almost each laboratory. The moderate costs

of the animals and ease of purchasing are among the important reasons for using this species in research. The size of the animals makes them very useful for various kinds of experiments. The numerous inbred strains available commercially guarantee that

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obtained results are repeatable and comparable with the results presented in the literature [44, 45]. Rats are relatively resistant to stress, tolerate well surgical procedures and are resistant to various infections [63]. Finally there is a considerable number of publications and manuals concerning the various aspects of anatomy and physiological functions in this species, which may be useful in planning new experimental procedures [63]. A large number of immunocytochemical agents, among other antibodies, can be used in this species and the results can be compared with others available in the literature [44]. These arguments explain the continuing great interest in using the rat in laboratory practice, including in neuroscience.

Ethical aspects of experimental and scientific work with laboratory animals

In each particular case of a planned experimental project, the acceptance of the Local Ethical Committee must be obtained, and the regulations outlined by the European Communities Council in the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" must be strictly complied with [5]. According to these regulations, vertebrate animals can be used in laboratory practice under restricted conditions only for the following purposes, as defined in the European Convention:

- avoidance or prevention of disease;
- detection, assessment, regulation or modification of physiological conditions in man, vertebrate and invertebrate animals or plants;
- protection of the environment;
- scientific research;
- education and training;
- forensic inquiries.

Each procedure should be selected in a way which enables researchers to use the minimum number of animals and cause the least pain, suffering, distress or lasting harm, and which is most likely to provide satisfactory results. In all possible cases the procedure should be performed under general or local anaesthesia or analgesia or by other methods designed to eliminate, as far as practicable, pain, suffering, distress or lasting harm throughout the procedure.

Pre-operative procedure

To provide anaesthesia in contemporary research laboratories, it is essential that adequate pre-operative preparations be made before beginning to anaesthetise an animal. Adequate pre-operative care will

reduce the incidence of many complications occurring during general anaesthesia. The most important is to use only healthy animals, which significantly decreases the risk associated with anaesthesia. Animals should be obtained at least 7 days prior to the intended experiment. This period is important for acclimatisation of the new animals to their new environment. During this period the metabolic and hormonal changes caused by the stress of transportation should return to normal [64]. Drinking water should be provided for the animals until approximately 60 minutes prior to induction of anaesthesia. Pre-anaesthetic fasting of rats is unnecessary since vomiting during induction does not occur in this species. The aim of premedication is to produce a calm and co-operative animal [17]. It is given in order to achieve:

- sedation and aid stress-free induction of anaesthesia;
- reduction of the amount of anaesthetic agents required to induce general anaesthesia;
- smoother induction of anaesthesia;
- blocking of the vaso-vagal reflex (the reflex slowing the heart rate which can occur as a result of endotracheal intubation and surgical procedures);
- decreasing of the volume of salivary and bronchial secretions that might block the airways.

Intraperitoneal injections are tolerated better than intramuscular injections because they cause less pain to the animal. Drugs that can be used to produce pre-anaesthetic sedation are shown in Table 1. Atropine in dose 0.05 mg/kg i/m, i/p or glycopyrrolate (0.5 mg/kg i/m) can be administered to reduce salivary and bronchial secretion and protect the heart from vagal inhibition [41].

General anaesthesia

General anaesthesia may be induced using a variety of drugs and ways of administration. A single drug can be given to achieve all the required elements of

Table 1. Sedatives, tranquilisers and other pre-anaesthetic medication for use in the rat

Drug	Dose rate
Diazepam [17]	2.5–5.0 mg/kg i/m, i/p
Midazolam [17]	5.0 mg/kg i/m, i/p
Ketamine [60]	50–100 mg/kg i/m, i/p
Fentanyl + Droperidol [19]	0.5 ml/kg i/m
Xylazine [21, 60]	1–5 mg/kg i/m, i/p

general anaesthesia, such as loss of consciousness, analgesia, depression of reflex activity and muscle relaxation [63]. Alternatively, a combination of agents can be given, each participating in the general effect. The benefit of such a method is that the side effects of anaesthetic agents can be reduced. The use of several drugs in combination, at a relatively lower dose, decreases the effect on all body systems occurring during anaesthesia with a single anaesthetic agent. The choice of a particular anaesthetic agent or anaesthetic technique will depend upon a variety of factors. Some of these should give consideration to the anaesthetic agent and its potential interactions with research protocols, others to its

ability to cause the demanded depth of anaesthesia [50]. The small body size of the rat makes intravenous induction of anaesthesia difficult and drugs are usually given by the intraperitoneal or intramuscular routes. Administration of an intravenous drug is limited by the small size of superficial veins. A further consequence of the small size of rats is that the volumes of anaesthetic agents are very small. Intermittent or continuous intravenous administration of anaesthetic agent requires cannulation of the femoral or subclavian vein (Fig. 1A–C). Especially for neurological purposes, it is important to know the effect of anaesthetics and anaesthetic techniques on cerebral circulation, metabolism and intracranial

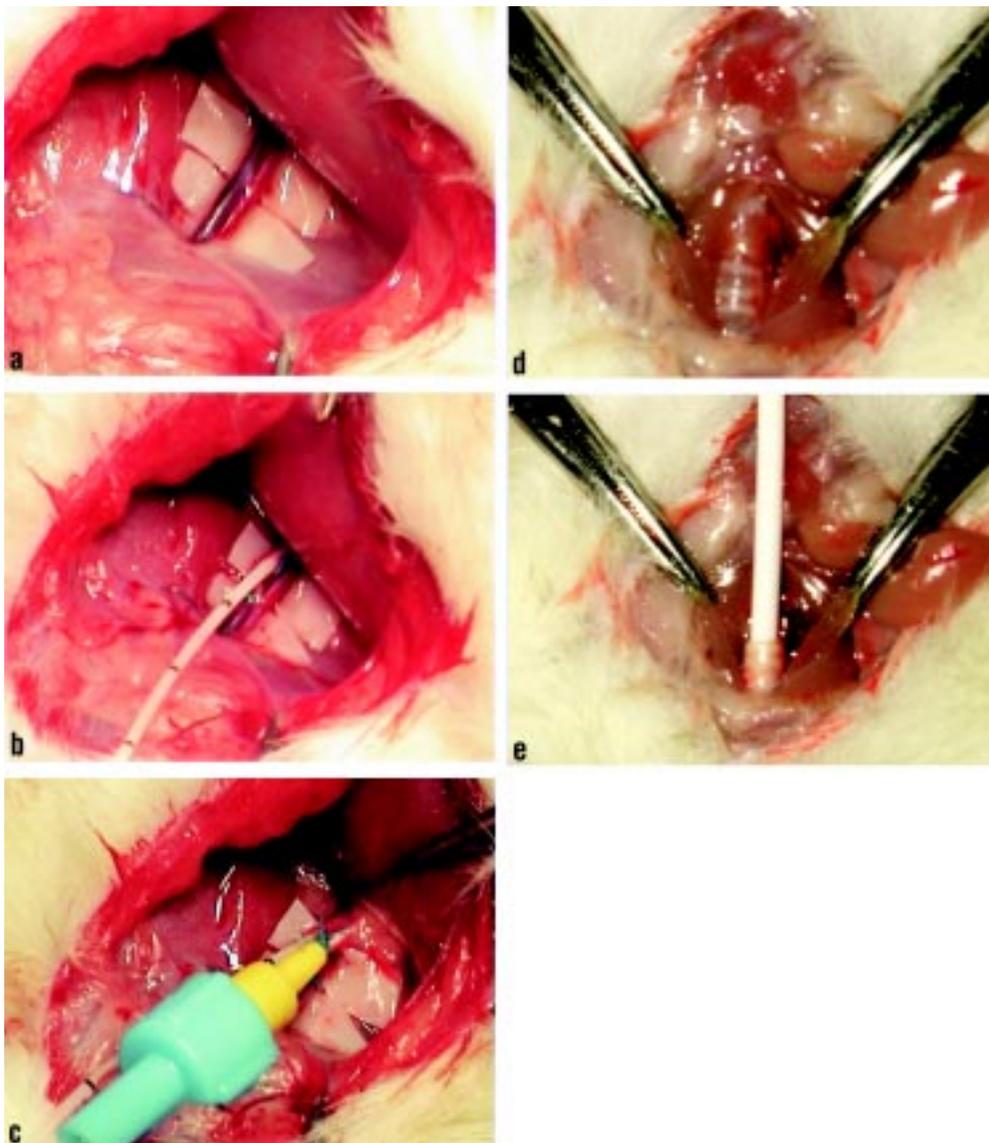


Figure 1. A, B, C. Cannulation of the femoral vein (for administration of the fluid and drugs) and artery (for the continuous recording of blood pressure); D, E. Tracheotomy in the rat and insertion of the intubation tube.

Table 2. Anaesthetic dose rates in the rat

Drug	Dose rate	Duration of anaesthesia (min)	Sleep time (min)
Ketamine + Xylazine [20, 23, 60, 66]	50–75 mg/kg + 10 mg/kg i/p	20–30	120–240
Ketamine + Midazolam [65]	50–75 mg/kg + 5 mg/kg i/p	20–30	120
Fentanyl + Medetomidine [24]	300 µg/kg + 300 µg/kg i/p	60–70	240–360
Thiopentone [1, 12, 65]	30 mg/kg i/v or 40–60 mg/kg i/p	10	15
Propofol [4, 9, 18, 55, 59]	5–10 mg/kg i/v	5	10

pressure in both normal and pathological conditions [36]. Intravenous anaesthetics in general cause a decrease in cerebral blood flow and metabolism [32–35, 51, 52, 55, 56, 59]. Among the intravenous anaesthetics ketamine may be unique, because it produces an increase in both cerebral blood flow and metabolism [7]. Anaesthetic drugs for use in the rat are presented in Table 2. In general, all inhalational anaesthetics can be considered to be more or less potent cerebral vasodilators, and thus possess the capability of increasing intracranial pressure [37–39, 42, 46, 47]. Volatile anaesthetics, with the possible exception of nitrous oxide, usually depress metabolism [6, 14, 36, 40, 53, 54, 57, 62]. Usage of inhalational anaesthetics requires flowmeters which may be unable to deliver low flow rates, as well as calibrated vaporizer (Fig. 2A). The potency of a volatile anaesthetic is indicated by its minimum alveolar concentration (MAC) value. This value is the alveolar concentration of an anaesthetic required to block the response to a specified painful stimulus in 50% of animals in a studied group [10]. The pharmacological properties of halothane, isoflurane and sevoflurane are presented in Table 3. Among the inhalational anaesthetics, isoflurane and sevoflurane appear to produce a moderate increase in

cerebral blood flow and a pronounced decrease in cerebral metabolism [56, 57]. Sevoflurane in concentration up to 1 MAC does not have a significant effect upon intracranial pressure and systemic circulation, not only in physiological conditions but also in the course of intracranial haematoma [25, 26]. The most suitable method of inducing anaesthesia by inhalational agents in rat is to use an anaesthetic chamber. Following induction of anaesthesia, the animal should be removed from the chamber and intubated, then anaesthesia maintained using the vaporiser and the ventilator [17, 22].

Muscle relaxants invoke paralysis of the skeletal muscles. They are used to obtain not only stable mechanical ventilation but also more suitable conditions for surgery. Drugs in this group include d-tubocurarine, pancuronium, vecuronium, mivacurium — they are presented in Table 4. These agents act by competing with acetylcholine for receptor sites at the neuromuscular junction. Their action can be reversed by increasing the local concentration of acetylcholine. This can be accomplished by the application of drugs such as neostigmine, which, through depressing cholinesterase-enzyme, increase the concentration of acetylcholine [16, 17, 62]. Administration of neuromuscular blocking agents requires artificial ventilation for the period of their action.

Table 3. Physiological properties of isoflurane, sevoflurane and halothane for use in the rat

	Isoflurane [30]	Sevoflurane [27]	Halothane [30]
Concentration for induction of anaesthesia (%)	4.0	6.0	4.0
Concentration for maintenance of anaesthesia (%)	1.5–3.0	2.3–4.6	1.0–2.0
Minimum alveolar concentration (%)	1.4	2.3	0.9

Table 4. Neuromuscular blocking agents

Drug	Dose rate	Duration of action (min)
d-tubocurarine [49]	0.1 mg/kg i/v	30–40
Mivacurium [58]	0.6 mg/kg i/v	2–6
Pancuronium [67]	0.1 mg/kg i/v	30–45
Vecuronium [2]	1.2 mg/kg i/v	20–30

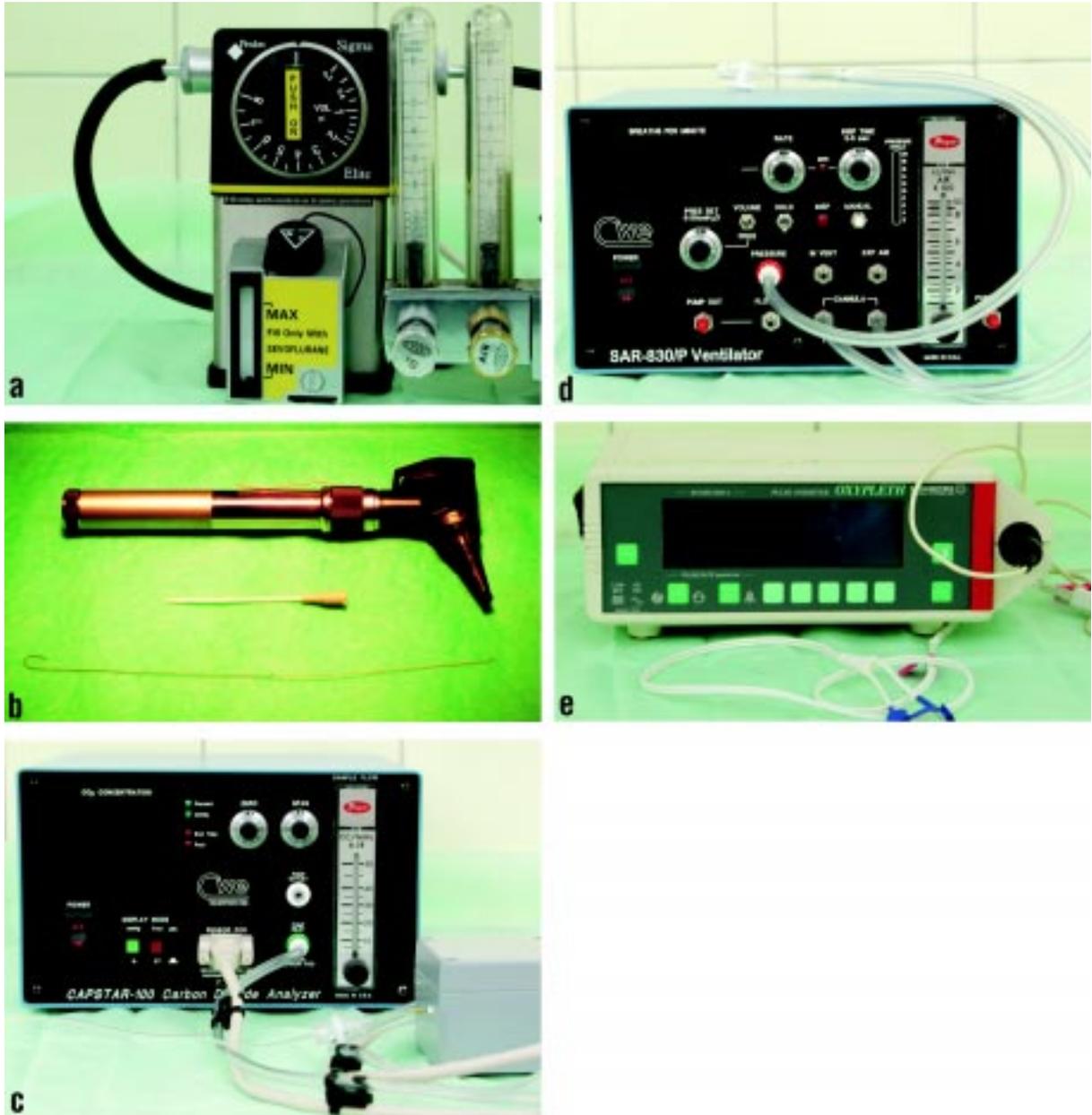


Figure 2. **A.** Apparatus for inhalational anaesthesia (the flowmeter for use of the oxygen and air, Sigma Elite Vaporizer for use of sevoflurane (Penlon, UK); **B.** Equipment for intubation of the rat (otoscope, Seldinger guide and cannula); **C.** End-tidal CO₂ Analyzer Capstar-100 (CWE, Inc. USA); **D.** Volume-controlled and pressure-controlled small animal ventilator SAR 830 (CEW, Inc. USA); **E.** Pulse oximetry in the rat (Novamatrix, USA).

Artificial ventilation

Many anaesthetic agents depress respiration and this can lead to hypoxia, hypercapnia and acidosis. To maintain blood carbon dioxide and oxygen concentration in physiological range, it is necessary to apply artificial ventilation. A mechanical ventilator allows for the precise control of the time of inspiration and expiration, of the gas volume delivered to the lungs and of airway pressure during inspiration (Fig. 2D).

Artificial ventilation requires tracheal intubation. Intubation of the rat can be performed using a special laryngoscope [11] or an otoscope. When using an otoscope, it is necessary to use an introducer, because the cannula will not pass through the lumen of the otoscope. A guide wire from a Seldinger catheter is a perfect tool since its tip is soft and flexible (Fig. 2B). The animal can then be intubated using a suitably sized (12–16 gauge) arterial or venous

cannula. To avoid unintentional intubation of one bronchus, a small piece of rubber tubing can be positioned around the catheter about 0.5–1.0 cm from the tip [17]. Alternatively, the insertion of the intubation tube is possible following tracheotomy (Fig. 1D, E).

Useful supporting equipment and its role during anaesthesia

The meaning of basic clinical observation, such as noting the colour of the mucous membranes and the colour of any blood that is shed at the operation field, even when using sophisticated equipment, is significant. These simple clinical observations can be performed by anyone and will always point to a disturbance in the animal's condition. Following the administration of anaesthetic agents it is necessary to determine that the required depth of anaesthesia has been reached. It is also helpful to observe the vital signs of the animal and the function of any anaesthetic equipment that is in use. More sophisticated techniques of assessment of depth of anaesthesia have been applied in humans, for example electroencephalography, and somato-sensory or auditory evoked potentials [28, 31]. These have not yet been widely applied in animals. Although measurements of mechanical parameters of respiration provide a practical indication of respiratory function, some effort must also be made to evaluate the sufficiency of lung gas exchange. A more sensitive method of measurement of blood oxygen saturation is pulse oximetry, which evaluates the percentage saturation of arterial blood, by detecting changes in the absorption of light across the tissues (Fig. 2E). Besides monitoring the saturation of haemoglobin with oxygen, the device can process the pulsatile nature of the signal, and from this calculate the heart rate. Application of a pulse oximeter gives three useful pieces of information. The degree of reduction of saturation of haemoglobin allows for evaluation of hypoxia due to respiratory depression, airway obstruction or anaesthetic device failure [15, 61].

Capnography is a continuous tracing which reflects the changing concentration of CO₂ during the respiratory cycle (Fig. 2C). Provided that the gas flow in the circuit is not altered capnograph readings will indicate trends during anaesthesia in small animals, and so are useful particularly in rats maintained using a mechanical ventilator. The following list describes some of the most important benefits of CO₂ monitoring [3]:

- disturbances in gas exchange, circulation and metabolism can be easily recognised by CO₂ monitoring;

- during the maintenance of anaesthesia CO₂ monitoring is an objective indicator of normoventilation, and thus enables a reduction in recovery time;
- quick detection of problems in the airway or malfunction of the ventilator or gas supply.

The most satisfactory method of monitoring the adequacy of lung gas exchange is to obtain blood samples and perform blood gas analysis. The result of blood gas analysis provides the partial pressure of oxygen and carbon dioxide and pH of the blood and, in addition, calculates the blood bicarbonate concentration and the base excess [29].

Direct recording of systemic blood pressure is an invasive procedure requiring arterial cannulation, which can be done following surgical exposure of a suitable artery. Invasive blood pressure monitoring has the advantage of providing a rapid response to changes in pressure. Pressure transducers for arterial pressure measurement are expensive items of equipment. Because its absolute asepsis is not required, they can be re-used successfully for a long period.

Monitoring of intracranial pressure is required especially in experiments on the central nervous system. The intraparenchymal intracranial pressure monitoring techniques are a relatively recent addition to the research and clinical armamentarium [8, 13]. Such devices as the Camino and the Codman monitor consist of fine fibre optic cable with a miniature transducer at the tip [43, 48]. These systems are easy to insert and only a little disruptive of brain tissue.

The problems which arise when anaesthetising rats are related to the small body size of these animals. Their high ratio of surface area to body weight makes them particularly susceptible to the development of hypothermia. Special attention should be given to the controlling of temperature and the monitoring of fluid balance. To record any change of rectal temperature that may occur during anaesthesia a clinical thermometer is necessary, but this needs repeated adaptation and substitution of instrument. It is especially important to avoid hypothermia in rats. The animal should be placed on a thermostatically feedback-controlled heating pad or a heating lamp can be used. Fluid replacements of most species are 40–80 ml/kg every 24 hours [17]. As a general guide, total fluid infusion of up to 10% of circulating volume per hour (7 ml/kg/h) is well tolerated by most animals [17]. If the animal is unable to receive fluid orally, it can be administered by the subcutaneous or intraperitoneal route. In rats (200 g), the approximate volume for fluid replace-

ment therapy by intraperitoneal or subcutaneous administration amounts to 5 ml [17].

In the postoperative period animals require special attention in the recovery area. It should be warm and quiet. The temperature of the surrounding area should be 27–30°C in the immediate postoperative period. Animals should be examined not less than twice a day. Observation of healing of wounds and surgical implants is also an important part of postoperative care, which may have an influence on the final results of the research.

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