Anesthetic protocols of macaque monkeys for functional MRI in Non-Human Primate Neuroimaging Neuroanatomy Project (NHPNNP)

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1. Induction

Macaque monkeys are initially administered intramuscular injection of atropine sulphate (20 μg/kg) to prevent hypersalivation during the following intubation. After 20 min, the animals are sedated with intramuscular injection of dexmedetomidine (4.5 μg/kg) and ketamine (6 mg/kg). A 22G indwelling needle was inserted into the tibial vein and caudal artery and connected to the line of physiological saline (incl. heparin) infused by natural instillation and by pressurized bag, respectively. The venous line is used for continuous infusion of anesthetics (dexmedetomidine) and the artery line for blood-gas analysis. After local anesthesia of laryngopharinx with a Xylocain spray, endotracheal tube with ID=3.5mm (1), coated with a Xylocain jelly, is inserted to the trachea. The tracheal tube is fixed to the animal's nasal and mandibular regions using a polyvinyl chloride string (Trinity Trach-Tube Ties, Fort Worth, TX). A steady respiratory ventilation is controlled using an anesthetic ventilator with a tidal volume of 10-15mL/kg and a ventilation rate of 0.2Hz. We use anesthetic ventilator which monitors the level of end-tidal carbon dioxide (EtCO2) and intratracheal pressure (Cato, Drager, Germany).

2. Maintenance

After the animal is fixed in an animal holder, anesthesia is maintained using inhalation of 0.6 % isoflurane (2) via a calibrated vaporizer with a mixture gas of air 0.75 L/min and O_2 0.1 L/min, and intravenous infusion of dexmedetomidine (4.5 μg/kg/hr) (3) using a syringe pump (TE-351, Terumo, Japan). Animals are warmed with blanket and were monitored throughout experiments for their rectal temperature (1030, SA Instruments Inc., NY, USA), peripheral oxygen saturation and heart rate (7500FO, NONIN Medical Inc., MN, USA). The arterial blood is sampled intermittently and analyzed for O_2 and CO_2 level. The blood gas data (PaO2, $PaCO_2$), $EtCO_2$ and used to adjust O_2 flow rate, ventilation rate (0.2 to 0.3 Hz) and a tidal volume (10-15mL/kg). The target values of O_2 and CO_2 in the arterial blood were 100 Torr (mmHg) and 40 Torr (mmHg) respectively. For diffusion imaging the level of isoflurane was increased to 1.0 % to reduce potential eye and head motion artefacts.

3. Withdrawal

After ending the experiment, infusion of DEX is discontinued and the venous and arterial catheters are taken out. Meanwhile, the concentration of isoflurane is kept the same and the respiration mode set to a mode of CPAP (continuous positive airway pressure) until animal's spontaneous respiration restarts. When respiration recovered, change the oxygen flow to 0 mL/min and set the inhaled gas only to air, and confirm that SpO_2 is kept over 95%. Then the intratracheal tube is removed and the respirator assist is discontinued. If there is excessive sputum or mucus, only the respirator is discontinued first, and intratracheal tube is not removed until the animal fully recovers respiratory function with cough reflex and returns to the housing cage or warmed cage for intensive care.

Notes

1) The optimal ID is different from the size of the macaque animal used. For your reference, we use ID=3mm for those with a body weight of 2-3kg, ID=3.5mm for 6-8kg and ID=4.0mm for 6-8mm.

2) The maintenance concentration of Isoflurane can be increased (e.g. 0.7%) depending on the animal's conditions such as mild anesthesia level or tachycardia. The low-dose Isoflurane is recommended since a single administration of DEX does not have full anesthetic effect and can result in movements of animals during scanning, which cause artifacts in fMRI data.

3) Use of DEX in the anesthesia protocol is based on the literature on relative preservation of functional connectivity in DEX-anesthetized humans (Guldenmund et al., 2017) and rodents (Pan et al., 2015). Compared with previous protocol of DEX in macaque (Zhao et al., 2015), we modified it to achieve a stable anesthetic condition with least adverse effect (i.e. cardio-suppression) by DEX and higher resting-state BOLD activities than those in isoflurane-only (1.0%) anesthesia in preliminary studies.

References

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