Anesthesia with alpha-chloralose in rats: it can be used for longitudinal fMRI studies

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Introduction: Animal experimentation in neurosciences requires the use of anesthetics for animal welfare and cooperation. Two of the most widely used anesthetics for functional magnetic resonance imaging (fMRI) of animals are Isofluorane (Iso) and Alpha-Chloralose (AC). Iso is a volatile drug shown to be suitable to obtain fMRI images at low concentrations (1). AC is an injectable anesthetic with strong functional-metabolic coupling. On the other hand it can create physiological problems so it has been used as non recoverable. Nevertheless there are studies were AC was used on human patients and others were AC was used to anaesthetize and recover dogs and cats (2,3). To our knowledge, the non-recoverable concept has not been challenged properly for fMRI. Here we present a protocol for AC anesthetic preparation together with a fMRI study that shows that AC can be used as a recoverable anesthetic and has no effects on the fMRI results when animals are reused. Furthermore two parallel behavioral studies on recovered rats (Rotarot and Tail flick tests) show no effect on their brain and motor function.

Methods: *Study design:* A group of 12 Sprage-Dawley rats with weights between 250g and 350g were studied over 18 days. A diagram of the study design can be seen in Figure 1. *Anesthesia:* Animals were induced in all cases with 5% Iso over 5 min. If the fMRI experiment was performed with Iso the mix was reduced to values between 1 and 1.3% inside the scanner (respirations per minute rpm = 63±5). For AC experiments, a tail cannulation was set after the 5 min. induction period and a bolus of 40mg/kg was then injected. A line for continuous flow of AC was maintained at 10-20mg/kg/h over the 1.5 hours of experimental time. Respiration rate dropped from 60±5rpm and stabilized round 40±5rpm at the end of the experiment. *Stimulation:* We performed electrical transcutaneous stimulation (10mA, 30 ms, 3Hz) on the right hindpaw. *fMRI Hardware:* FMRI experiments were performed on a 4.7 T BRUKER Biospec scanner, horizontal magnet (400mT/m) with a free bore of 40 cm, equipped with an actively RF-decoupled coil system. A whole-body birdcage resonator enabled homogenous excitation together with a 4 channel rat transmit/receive surface coil. *fMRI data:* Gradient-Echo Echo Planar Imaging sequence (GE-EPI), 2 excitations, TEef=24.4ms, TR=4s, in-plane resolution 390×390µm, 1mm slice thickness, matrix 64×64, FOV of 25x25mm. fMRI experiments were always followed by anatomical images. Anatomical data was acquired with a RARE sequence, TE=51ms, TR= 3000ms, in-plane resolution 9.7×9.7µm, 0.5 thickness, matrix 256 ×256, FOV of 25x25mm. *Data analysis:* Functional data analysis was performed using BrainVoyager QX V1.10 (Brain Innovation B.V. Netherlands). Slice time correction, motion correction, high pass temporal filtering and 2D Gaussian smoothing of data (2 pixel kernel) was performed. Single subject quantification of the activated voxel groups and time profiles of the activated structures were obtained by a custom written analysis program in IDL. *Physiological monitoring:* Temperature was monitored and kept at 37±1˚C with hot flowing water into the cradle. Respiration rate was constantly monitored with a pressure pad under the rat skin.

Results: Figure 2 shows the representation of hindpaw electrical stimulation for a single animal after the four fMRI experiments. The Cingulum is signaled with a blue arrow and appears clearly for all the experiments. The average tail flick test times were not affected the day after the experiments by any of the drugs (Table 1). All animals passed the Rotarot test in the 24 occasions it was performed. All 12 animals survived the protocol and showed no changes in their behavior. Animals gained weight over the 18 days of study (3.97±1g gain per day per rat) and were alive and showing no problems 5 days after the project was finished (day 23).

Discussion & Conclusions: We believe that some of the reasons why AC was unsuccessful before are the large amounts of anesthesia used. We reduced this factor to a minimum where animal would be anaesthetized but never show auditory reactions or jerk movements. A second reason for animal death would be the uncompleted solution of AC when mixed with a buffer to produce the anesthesia. Initial attempts where the buffer was not completely dissolved always produced respiratory problems and generally the death of the animal. With this protocol linear studies and reuse of animals is now possible with AC.

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References: (**1**) Hess A., 2006Eur J Pain. 2007 Jan;11(1):109-19. Epub 2006 Mar 6. (**2**). Williams HL., Psychopharmacologia. 1969;15(1):28-38. (**3**) Gilad S., Vet J. 2006 Jul;172(1):109-13.

Figure 1

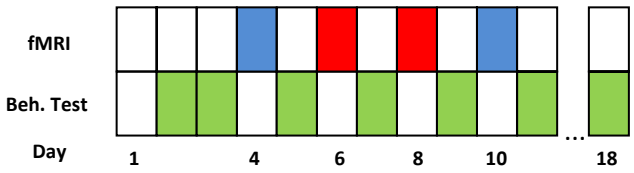

Figure 1. Protocol of the recovery study. This figure shows the protocol of this recovery study using AC and ISO as anesthetics. The days where fMRI experiments were performed appear in the top row (red boxes for AC and blue boxes for ISO). The days when behavior tests were performed (Rotarot and Tail flick test) appear in the middle row (green boxes). The number of experimental days appear in the bottom row.

Table 1: Tail flick test response times

	Resp. (s)
Before	6,44±0,7
ISO 1	5,98±0,3
AC 2	5,82±0,4
AC 3	6,22±0,5
ISO 4	6,24±0,5

Table1. Tail flick test time scores. This table shows the average time necessary to obtain a response from the Tail flick test. "Before" corresponds to the measurements performed before the fMRI studies started (Days 1 and 2). Then other four rows correspond to results obtained on the day after of the experiment marked on the left (Days 5,7,9 and 11 respect.).

Figure 2

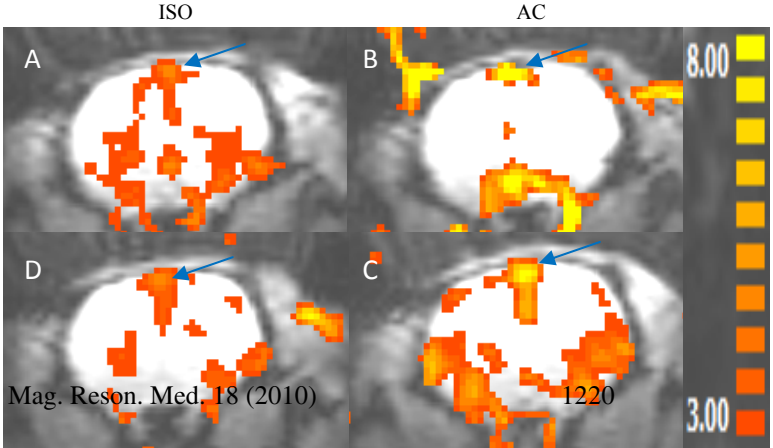

Figure 2. fMRI images of hindpaw electrical stimulation. These images present axial slices situated 0.5 mm rostral to Bregma passing through the Cingulate cortex. BOLD representation is produced by electrical stimulation of the right hindpaw. Blue arrows point at the activation in the Cingulate. The slice presented is the same in all cases. A and D are images obtained from experiments that used ISO as an anesthetic. B and C are images corresponding to experiments that used AC as anesthetic. All images were threshold to a minimum Z>3. The color bar on the right shows the significance of our activation.