# Etomidate: A novel anesthetic of choice for functional magnetic resonance imaging in mice

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**TARGET AUDIENCE:** The presented data are of interest for scientists involved in functional imaging in preclinical disease models or for basic neuroscience application

**PURPOSE:** Genetic mouse models of neurodevelopmental disorders, such as autism spectrum disorders (ASD), have become important tools to investigate pathophysiological consequences of specific genetic liabilities identified in patients(1). Functional MRI (fMRI) applied to these mouse models holds the promise to reveal pathological alterations at the macroscopic neurocircuitry level and to identify potential imaging biomarkers for clinical studies. Such studies across different ASD models, preferably in a longitudinal design, require a reliable and non-terminal anesthesia protocol which is applicable to a broad range of mouse lines. Isoflurane (ISOF) and medetomidine (MEDE), i.e. two anesthetics widely used in rodents (2,3) have shortcomings in that in mice ISOF drives cerebrovascular perfusion to a ceiling at doses required to maintain anesthesia in freely-breathing animals whereas MEDE results in a lack of sedation in numerous mouse lines. Here, we set out to establish a newly anesthesia protocol based on etomidate (ETOM), i.e. an anesthetic that binds to a specific pocket on the β-subunit of the $GABA_A$ receptor (4). For validation and comparison to ISOF and MEDE, we used continuous arterial spin labeling (CASL)-based perfusion MRI to assess cerebrovascular reserve capacity (CVRC) by administering the carbonic-anhydrase blocker acetazolamide (5), performed pharmacological fMRI (phMRI) studies upon an intervention with olanzapine and tested sensory stimulation using blood-oxygen-level-dependent (BOLD) fMRI (6).

**METHODS:** Studies were conducted in C57Bl6, BTBR and CD1 mice (25-35g) with n≥3 individuals per group. In all mice, anesthesia was induced with ISOF (2-3%) lasting for ~8min before transition to the respective test protocols, i.e. (i) ISOF 2% (in air:$O_2$, face-mask), (ii) MEDE s.c. 0.6mg/kg/h following a priming dose of 0.3mg/kg (iii) ETOM i.v. 0.75-1.5mg/kg/min following a priming dose of 4mg/kg/min. Appraisal of the anesthesia protocols consistet of perfusion MRI for assessing CVRC in terms of absolute perfusion at rest and change upon i.v. or i.p. dosing of 30mg/kg acetazolamide. Perfusion-based phMRI was performed with olanzapine (3mg/kg or vehicle i.p.). BOLD fMRI was carried out upon sensory stimulation of the hindpaw. For BOLD fMRI experiments animals were paralyzed and ventilated and a pair of electrodes were inserted subcutaneously into the left hindpaw as described elsewhere (6). **Perfusion-MRI** was carried out on a BioSpec 9.4T/20 MR system (Bruker BioSpinMRI, Germany) equipped with a volume resonator for transmission and a surface coil for reception. Perfusion imaging was performed based on continuous ASL with centred-RARE readout (TR/TE=3s/5.4ms, RARE=32, 128x64 matrix, 0.6mm slice thickness, 8 slices, 2 averages, 3s labeling, 0.4s post labeling delay). For registration to an anatomical template and region-of-interest wise absolute quantification of perfusion, $T_2$-weighted anatomical images and $T_1$-maps were acquired followed by a series of CASL images comprising either 3 (basal perfusion) or 12 volumes (time series). For phMRI analysis perfusion values for each ROI were normalized to plane-wise brain-mean perfusion. **BOLD-fMRI** experiments were conducted on a Biospec 94T/30 MR system (Bruker BioSpinMRI, Germany). A receive-only cryogenic phased array coil (Bruker BioSpin AG, Switzerland) was used in combination with a room temperature volume resonator for transmission. Data were acquired using a gradient-echo echo-planar imaging (GE-EPI) sequence: FOV=16x7mm², MD=80x35, voxel dimension of 0.2x0.2mm², flip angle (FA)=60°, TR/TE=1s/12ms, 1 average. The stimulation paradigm consisted of a block design with four cycles of a 20s stimulus period (2mA, 5Hz) interleaved with periods of 60s of rest. For analysis of fMRI data, spatial preprocessing and generation of statistical parametric maps (activity maps) on the basis of a general linear model (GLM) and z-statistics were performed by combining modules of AFNI (http://afni.nimh.nih.gov) and FSL (http://fsl.fmrib.ox.a.uk).

**RESULTS AND DISCUSSION:**

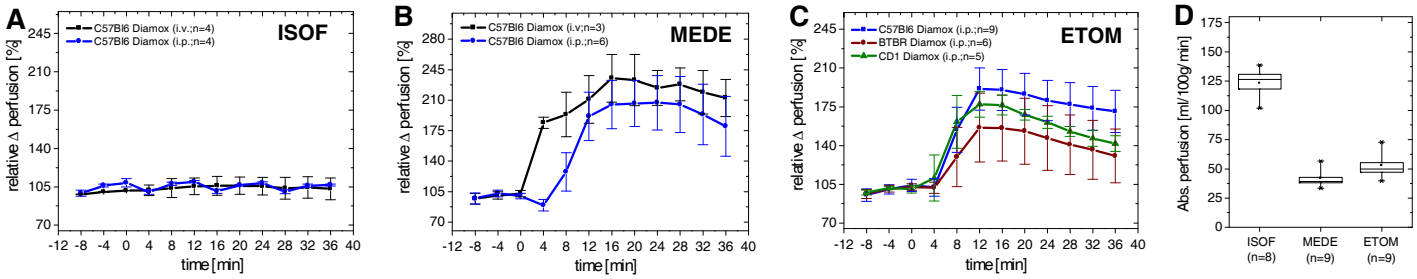


**Fig. 1**: **(A-C)** Cerebral perfusion change (total brain) in freely breathing C57Bl6 upon injection of acetazolamide as a measure of CVRC under 3 anaesthetics. Under ETOM (C57Bl6, BTBR, CD1) acetazolamide was administered i.p. (tail vein used for ETOM). **(D)** Absolute perfusion values at rest for all protocols in C57Bl6. CVRC is severely compromised under ISOF-2% but preserved under MEDE and ETOM.

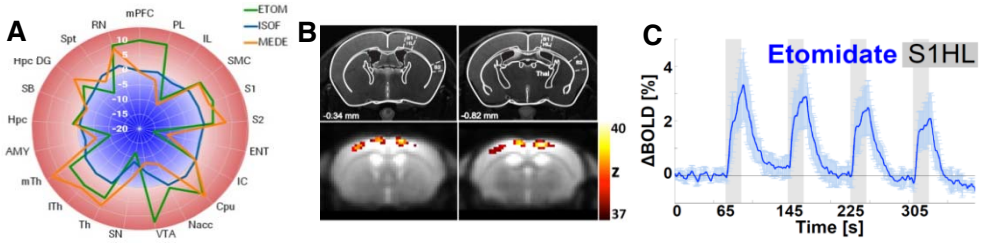


**Fig. 2**: **(A)** phMRI results for three different anesthesia protocols: radar plot showing perfusion changes induced by acute dosing with olanzapine (n=8, per group) in a subset of brain regions (in percent vs. vehicle, scaled from -20 to +15). **(B)** Sensory fMRI under ETOM in C57Bl6 mice. *Upper panel*: Anatomical images with regions-of-interest in the contralateral primary somatosensory hind limb cortex (S1HL) *Lower panel*: Statistical z maps at the group level (n=5) for two planes covering the S1HL. **(C)** Stimulus-evoked BOLD signal changes in S1HL expressed as percentages relative to baseline during interval block stimulation of left hind paw (n=5, mean ±SD).

While ISOF is routinely used for fMRI studies in freely-breathing rats (7) the same protocol applied here in mice compromised CVRC as demonstrated by the absence of a change in brain perfusion upon injection of the vasodilatory drug acetazolamide (Fig, 1A). By contrast, CVRC under MEDE and ETOM is preserved (Fig 1B and C, respectively). The lack of CVRC in freely-breathing mice under ISOF may be attributed to its well-documented strong vasodilatatory effect that lead to maximaly dilated arteries and caused high cerebral perfusion at rest and abrogated cerebral autoregulation (8), whereas perfusion under MEDE and ETOM stayed within a physiological range (Fig.1D). However MEDE can not be universally applied to mice due to the absence of its sedative effect in certain lines (e.g. BTBR and CD1), whereas, the novel anesthesia protocol based on ETOM led to a deep and stable anesthesia across a broad range of strains with intact CVRC (Fig1C, C57Bl6, BTRB and CD1). In line with the CVRC data, acute dosing with olanzapine in C57Bl6 mice elicited region-specific changes under MEDE and ETOM anesthesia but did not induce any significant modulation of perfusion under ISOF. The olanzapine-induced perfusion changes under MEDE and ETOM show similarities but also regional differences - possibly due to the anesthetic's different modes of action. Perfusion changes were observed in regions of the cortico-striatal-thalamic loops (i.e. caudate putamen, CPu; thalamus, Th; medial prefrontal cortex, mPFC), the primary circuitry enganged by antipsychotics. Sensory fMRI experiments performed under ETOM revealed strongest BOLD changes in the S1HL region of the order of 3% and correlated with the stimulation paradigm. **CONCLUSION:** We have developed a new anesthesia protocol based on etomidate and demonstrated its suitability for functional MRI studies in mice. This novel protocol preserves CVRC with basal perfusion values in a physiological range. Moreover it allows for longitudinal studies and is applicable to a broad range of mouse lines, thus making it the anesthesia-of-choice for cross-comparisons between different mouse models of brain disorders.

**REFERENCES:** **[1]** Petrinovic & Kuennecke (2014) Psychopharmacology (Berl). 231(6):1167-89 **[2]** Reese et al., (2000) NMR Biomed. 13(1):43-9. **[3]** Weber et al., (2006) Neuroimage. 29(4):1303-10. **[4]** Li et al., (2010) J. Biol Chemistry 285,12, pp. 8615–8620 **[5]** Mueggler et al., (2002) J Neurosci. 22(16):7218-24. **[6]** Schroeter et al., Neuroimage (2014);94:372-384. **[7]** Bruns et al., (2009) Magn Reson Med 61:1451–1458 **[8]** Wang et al., (2010), Exp Brain Res. 207(3-4):249-58.
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