Animal Welfare Aspects of different Bleeding Methods in Mice

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Aim:
According to the new EU Directive 2010/63/EU having the choice between different methods, the method causing least pain and suffering should be chosen. The German Society of Laboratory Animal Science (GV-SOLAS) recommends 5 different bleeding methods for mice. The question is, which one causes least pain and suffering when performed by a well trained technician.

Methods:

Methods to be tested:
- Retrobulbar puncture with large capillary (ø 1.5 mm)
- Retrobulbar puncture with small capillary (ø1.2 mm) under anaesthesia
- Puncture of tail vein
- Puncture of Vena saphena
- Puncture of Vena facialis
- Puncture of Venous angle under anaesthesia

For each methods 12 female BALB/cOlaHsd-mice 8 weeks old Experienced technicians for each method from all over Germany
Amount of blood to be sampled 75 µl

Histology of puncture sites immediately after puncture, after 3 days and after 2 weeks

Experimental design:

Induction of Anaesthesia Sampling method to be tested Home cage observation Corticosterone determination Open Field Test

if necessary Time 0 for 10 minutes after 15 minutes for 5 minutes

Conclusion:
Puncture of the retrobulbar venous plexus with the large capillary or of the V. facialis are the methods, which only need a few seconds to perform, but the puncture of the V. facialis apparently causes pain as stress indicated by a reduced home cage activity, a higher corticosterone increase and reduced open field activity. The corticosterone increase is moderate following the the retrobulbar puncture with large or small capillary as well as following the puncture of the V. saphena. The home cage activity and the open field activity is severely reduced after puncturing the V. saphena and the venous angle. As there were no differences in the pathological finding immediately and 3 days after puncture, only the differences after 2 weeks are shown with less pathological finding following the retrobulbar puncture no matter which capillary is used, while the puncture of the V. saphena and the puncture of the venous angle caused pathological at about 10 %. According to these results the puncture of the retrobulbar venous plexus can still be recommended either with or without anaesthesia depending on the experience of the technician.

Results:

Figure 1: Necessary time to collect 75 µl of blood

Figure 2: Activity in home cage scored 1 – 7
(1 = non active, 7 = very active)

Figure 3: Corticosterone increase after 15 minutes

Figure 4: Open field behaviour: Distance run within 5 minutes

Figure 5: Pathological findings (%) after 2 weeks

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