The 3 Rs

• Reduction
• Replacement
• Refinement

No specific research program on 3Rs and imaging in laboratory animals
Non-Invasive Methods: Investigation of Airways Diseases by MRI in Rats

Nicolau Beckmann, Stefan Zurbruegg and François-Xavier Blé
Novartis Institutes for BioMedical Research (NIBR), Basel, Switzerland
Database search «Imaging & 3Rs»

- **UCD Center for Animal Alternatives** has developed several biblioguides on animal experimentation. One of them concern non-invasive imaging in research.

- [http://www.vetmed.ucdavis.edu/Animal_Alternatives/imaging.html#overview](http://www.vetmed.ucdavis.edu/Animal_Alternatives/imaging.html#overview)
“Reductions by almost 80 per cent”

Non-invasive imaging techniques are methods to observe an organism (a mouse, for example) without having to penetrate the organism. Thus, these are gentle methods that have a number of advantages in regard to animal testing.
Is there really a need to investigate the 3Rs in imaging?

With the exception of the MRI research sponsored by the 3R Foundation, no specific research program is, to my knowledge, running in Switzerland on 3Rs and imaging in laboratory animals.
Bioluminescence

- More HE
- Reduction of number
- Short image times
- Non-toxic

- General anesthesia

Adapted from Hudson, M., Animal Welfare 14, 303, 2005
Fluorescence (visible & NIR)

- More HE
- Reduction of number
- Short image times
- No anesthesia, substrate or radioactivity

- Deeper tissue penetration by surgery (skin-flaps)

Adapted from Hudson, M., Animal Welfare 14, 303, 2005
Quantum dots (visible)

- More HE
- Reduction of number
- Short image times
- No anesthesia

- Toxic to cells (to be proved)

Adapted from Hudson, M., Animal Welfare 14, 303, 2005
PET imaging

- More HE
- Reduction of number
- Less severe blood sampling
- Sacrifice avoided
- Toxic nature of reporter gene
- Radioactive substrate
- General anesthesia
- Stereotaxic frame for CNS studies

Adapted from Hudson, M., Animal Welfare 14, 303, 2005
MRI scanning

- More HE
- Reduction of number
- Replacement of invasive procedures

- High field strength > excessive heating of body
- General anesthesia (?)
- Contrast agents may interfere with biological processes

Adapted from Hudson, M., Animal Welfare 14, 303, 2005
Magnetoencephalography in Humans

- Replacement of animals
- n/a

Adapted from Hudson, M., Animal Welfare 14, 303, 2005
Optical Topography (NIR light)

- Replacement of animals
- n/a

Adapted from Hudson, M., Animal Welfare 14, 303, 2005
Imaging and the 3Rs (1)

- **Replacement**
  - MEG as an *alternative to primate research* in visual attention
  - OT for investigating *brain function in human* instead of animals

- **Reduction**:
  - **Less animals are used**
    - More pain? (→ room for refinement)
  - **Health status** and data variability
Imaging and the 3Rs (2)

- **Refinement**
  - Humane Endpoints: could we do more?
  - Longitudinal studies: less animals=more pain?
  - Best practices in Laboratory Animal Science: imaging as training assessment
Humane Endpoints

Tumor Response Assessment Is More Robust With sequential CT Scanning Than External Caliper Measurements

Conclusion
Tumor size measurements by means of CT from PET/CT were more reliable than caliper measurements because of their smaller variance, allowing earlier assessment of response. It is suggested that CT imaging-based methods of assessing tumor response replace traditional caliper-based measurements, much as CT has become a standard for assessing tumor response in humans.

Takayoshi Ishimori MD, PhD, Mitsuaki Tatsumi MD, PhD and Richard L. Wahl MD (Academic Radiology 12, 2005, 776)
Survival prediction with BLI

Taken from « Biophotonic imaging and the reduction of animal usage in experimental research
Francis, K.P. NC3Rs#1 Biophotonic imaging sept 2005
Quantitative Molecular Imaging of Murine Sepsis: a Predictive Model

Table 1A. Sensitivity and Specificity of a Threshold Slope of 0.05

<table>
<thead>
<tr>
<th>Slope</th>
<th>Dead Mice</th>
<th>Survivor Mice</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 0.05</td>
<td>35 (92.1%)</td>
<td>1 (4.8%)</td>
<td>36</td>
</tr>
<tr>
<td>&lt; 0.05</td>
<td>3 (7.9%)</td>
<td>20 (95.2%)</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>21</td>
<td>59</td>
</tr>
</tbody>
</table>

Carolyn Bellinger-Kawahara, Kevin P. Francis, Alan Hubbard, Anthony F. Purchio, and Pamela R. Contag (unpublished data)
Humane Endpoints

• Published studies on finding HE with the aid of imaging techniques are quite rare

Need for research in the Refinement of the 3Rs
Longitudinal Studies

• Radiation doses
  – MicroCT
  – MicroPET & SPECT

• Anesthesia
  – Repetitive
  – Alternative: Awake animal (fMRI)?

• Health Status
  – Clean or dirty animals?
Radiation Doses & CT imaging

– Single measure: LD50/30: 5.0 to 7.6 Gy depending of strain, age, etc…

– Repeated measures: Mouse neutralizes 0.25 to 0.5 Gy per day if exposed daily

– Interferences with tumor treatment (drug or gene therapy)

• Image resolution of live animals will be limited by radiation dose, especially important for longitudinal studies.

Ford al. Med Phys, 30, 2869, 2003
Radiation Doses & CT imaging

• Ultrafast CT image acquisition vs « step & shoot » conventional protocol
  – A decrease by a factor 6.5 of tumor dose
  – Received dose is 10 times lower than the ones which may affect tumor growth inhibition

• No radiation evaluation from additional injected radionuclides for specific microSPECT/CT protocols.

Carlson et al. Mol Imaging Biol, 9, 78, 2007
PET imaging with fluorine-18 labeled compounds

Monte Carlo simulations of absorbed dose in a mouse phantom from 18-fluorine compounds

Richard Taschereau and Arion F. Chatziioannou
The Crump Institute for Molecular Imaging, Department of Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA, 700 Westwood Boulevard, Los Angeles, California 90095

• Depending of the tracer, high doses are delivered to specific organs (bladder, heart, kidney, bone, bone marrow, tumor xenograft)

• Voiding of bladder should become standard practice.
Table II. Calculated absorbed dose for a 7.4 MBq injection.

<table>
<thead>
<tr>
<th>Structure name</th>
<th>FDG (mGy/7.4 MBq)</th>
<th>FLT (mGy/7.4 MBq)</th>
<th>Fluoride ion (mGy/7.4 MBq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder wall (low-res)</td>
<td>4018</td>
<td>3130</td>
<td>2531</td>
</tr>
<tr>
<td>Bladder wall (hi-res)</td>
<td>3915</td>
<td>3050</td>
<td>2466</td>
</tr>
<tr>
<td>Body</td>
<td>106</td>
<td>96</td>
<td>77</td>
</tr>
<tr>
<td>Brain</td>
<td>98</td>
<td>89</td>
<td>94</td>
</tr>
<tr>
<td>Cranium</td>
<td>30</td>
<td>30</td>
<td>457</td>
</tr>
<tr>
<td>Heart</td>
<td>425</td>
<td>96</td>
<td>61</td>
</tr>
<tr>
<td>Intestine wall</td>
<td>29</td>
<td>81</td>
<td>19</td>
</tr>
<tr>
<td>Kidneys</td>
<td>195</td>
<td>104</td>
<td>117</td>
</tr>
<tr>
<td>Lower limbs</td>
<td>26</td>
<td>22</td>
<td>473</td>
</tr>
<tr>
<td>Liver</td>
<td>42</td>
<td>95</td>
<td>58</td>
</tr>
<tr>
<td>Lungs</td>
<td>67</td>
<td>44</td>
<td>79</td>
</tr>
<tr>
<td>Pancreas</td>
<td>100</td>
<td>96</td>
<td>56</td>
</tr>
<tr>
<td>Ribs</td>
<td>38</td>
<td>35</td>
<td>206</td>
</tr>
<tr>
<td>Skin</td>
<td>19</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>Bone (spine)</td>
<td>36</td>
<td>32</td>
<td>560</td>
</tr>
<tr>
<td>Marrow (low-res)</td>
<td>74</td>
<td>67</td>
<td>306</td>
</tr>
<tr>
<td>Marrow (hi-res)</td>
<td>...</td>
<td>...</td>
<td>488</td>
</tr>
<tr>
<td>Spleen</td>
<td>96</td>
<td>88</td>
<td>52</td>
</tr>
<tr>
<td>Stomach wall</td>
<td>32</td>
<td>37</td>
<td>26</td>
</tr>
<tr>
<td>Testes</td>
<td>118</td>
<td>106</td>
<td>64</td>
</tr>
<tr>
<td>Thyroid</td>
<td>105</td>
<td>95</td>
<td>37</td>
</tr>
<tr>
<td>Tumor</td>
<td>223</td>
<td>488</td>
<td>...</td>
</tr>
<tr>
<td>Vas deferens</td>
<td>221</td>
<td>181</td>
<td>136</td>
</tr>
</tbody>
</table>
Fig. 6. Normalized isodose map (mGy/MBq) for the bladder wall in an $^{18}$FDG study as a function of the time and amount of bladder voidance. The time is given in minutes following injection and voidance is expressed as a fraction between 0 (full bladder) and 1 (empty bladder).
Anesthesia versus Awake state


Awake but curarized animals have a higher, more widespread BOLD signal change following forepaw stimulation, but the more widespread, non-specific activation makes the interpretation much more difficult. Definitely the problems and difficulties for interpretation that will be encountered using awake animals will mostly outweigh the advantages of the higher signal change.
fMRI in Awake state

- Controlling for motion artifacts
- Controlling for stress
- Eliciting “natural” behavior
- Identifying discrete behavioral responses
- Noise
Animal restrainer for fMRI

Ludwig et al., J Neuro Meth 132, 125, 2004
Controlling for motion artifacts

![Graphs illustrating head motion in the horizontal and vertical planes with standard deviation bars for Day 1 and Day 5.](image-url)
Controlling for stress
Best practices in LAS

- Wiles et al. propose to use biophotonic imaging as a training aid to injection routes in laboratory rodents (Lab Animals 2007, 41, 321).
- Ideal for teaching but need alive animals.
- Contribute to assess on-line effectiveness of complex / novel route of administration.
Injection routes & BLI

Route of administration

- Subcutaneous
- Intranasal
- Oral gavage
- Intra-peritoneal

(A) Immediately after injection
(B) 45 minutes after injection
Injection routes & BLI

Route of administration
- Oral gavage
- Intrapерitoneal

Anaesthetized

Immediately after cervical dislocation

Luminescence (photons s⁻¹ cm⁻² sr⁻¹)

10 x 10⁶
8 x 10⁶
6 x 10⁶
4 x 10⁶
2 x 10⁶
Animal health status & Imaging

• It is known that the microbiological status of an animal impact on the research results. Therefore the Reduction of the 3Rs could be negatively affected by increased variability of results.
  – See for review: « Implications of infectious agents on results of animal experiments », Laboratory animals 33 (suppl1), S1:39, 1999.

• Should we care about it in small animal imaging?
Conclusions

• Imaging techniques are an important contribution to the 3Rs.
• Refinement is a topic for more research in the domain of imaging.
• Health status in imaging projects is a real challenge.
• Imaging techniques may help in LAS training
More information

• See [http://wiki.epfl.ch/imaging-3rs](http://wiki.epfl.ch/imaging-3rs) for additional information and copy of my presentation

THANK YOU FOR YOUR ATTENTION
Overheating in fMRI?

- See Trakic et al paper in Phys Med Biol 49, 5547, 2004. Numerical modeling of thermal effects in rats due to high-field magnetic resonance imaging (0.5 – 1 GHz)

<table>
<thead>
<tr>
<th>Frequency (MHz)</th>
<th>$T_{\text{max}}$ (°C)</th>
<th>$T_{\text{avg}}$ (°C)$^a$</th>
<th>$\Delta T_{\text{max}}$ (°C)</th>
<th>$\Delta T_{\text{avg}}$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>36.96</td>
<td>36.73</td>
<td>0.26</td>
<td>0.03</td>
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<tr>
<td>723</td>
<td>37.41</td>
<td>36.78</td>
<td>0.71</td>
<td>0.08</td>
</tr>
<tr>
<td>1000</td>
<td>38.91</td>
<td>37.04</td>
<td>2.21</td>
<td>0.34</td>
</tr>
</tbody>
</table>

$^a$ Rat body temperature: 36.7 °C.
Table 1. Compiled from a Medline (1997–2001) search using the key words anaesthesia and imaging. A total of 293 papers were retrieved. The percentage of investigations using each anesthetic drug is listed.

<table>
<thead>
<tr>
<th>Anaesthetic drug</th>
<th>%</th>
<th>Anaesthetic drug</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acepromazine</td>
<td>10</td>
<td>Ketamine</td>
<td>6</td>
</tr>
<tr>
<td>Alphadalone–alphaxalone (Saffan)</td>
<td>2</td>
<td>Ketamine–midazolam</td>
<td>1</td>
</tr>
<tr>
<td>Alpha chloralose</td>
<td>4</td>
<td>Ketamine–xylazine</td>
<td>7</td>
</tr>
<tr>
<td>Barbiturate (unidentified)</td>
<td>1</td>
<td>Methohexitol</td>
<td>1</td>
</tr>
<tr>
<td>Chlora hydrate</td>
<td>2</td>
<td>Methoxyflurane</td>
<td>1</td>
</tr>
<tr>
<td>Droperidol–fentanyl (Innovar)</td>
<td>1</td>
<td>Morphine</td>
<td>1</td>
</tr>
<tr>
<td>Enflurane</td>
<td>3</td>
<td>Morphine–alpha chloralose</td>
<td>1</td>
</tr>
<tr>
<td>Ether</td>
<td>3</td>
<td>Pentobarbital</td>
<td>16</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>1</td>
<td>Propofol</td>
<td>6</td>
</tr>
<tr>
<td>Halothane</td>
<td>17</td>
<td>Spinal or intrathecal anaesthesia</td>
<td>1</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>13</td>
<td>Thiopental</td>
<td>2</td>
</tr>
</tbody>
</table>

NMR Biomed 16, 459, 2003
Fig. 10. Photographs showing a conscious dam restrained in the device used for functional magnetic resonance imaging. A cradle of the dam’s pups is positioned to allow access to the teats for suckling. © 2005 The Society for Neuroscience.

Functional magnetic resonance imaging in conscious animals