Outline

• Suffering Model

• Some measure of extent of animal distress/suffering

• USA Comparison

• Examples of effects on research

• Conclusions
PAIN/DISTRESS/SUFFERING MODEL

PAIN OR ANXIETY OR FEAR OR DISCOMFORT

$\times \{[\text{TIME}] + [\text{INTENSITY}]\}$

acute DISTRESS chronic

COGNITIVE FILTER

SUFFERING
Amount of Distress

• Regulatory attempts to assess the level of distress in laboratory animals are rudimentary. The USDA does not even have a definition for distress.

• In Canada (and NZ, Switzerland, the Netherlands and the UK) the regulators have attempted to measure the extent of moderate and/or severe pain and distress.
Canadian Numbers-1
P&D Classes

- B: No pain or distress
- C: Minor pain or distress
- D: Moderate to severe pain or distress
- E: Severe pain or distress
28.7% experience moderate/severe P&D, and 5.2% experience severe P&D. About 44% of this P&D in testing area.

<table>
<thead>
<tr>
<th></th>
<th>BASIC RES.</th>
<th>MED. RES.</th>
<th>REG. TEST.</th>
<th>DRUG DEV.</th>
<th>EDUC.</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>333,393</td>
<td>51,813</td>
<td>70,017</td>
<td>29,034</td>
<td>57,716</td>
</tr>
<tr>
<td>C</td>
<td>536,995</td>
<td>94,565</td>
<td>56,335</td>
<td>10,605</td>
<td>10,806</td>
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<tr>
<td>D</td>
<td>120,236</td>
<td>116,717</td>
<td>146,980</td>
<td>29,097</td>
<td>2,406</td>
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<tr>
<td>E</td>
<td>12,694</td>
<td>5,466</td>
<td>74,236</td>
<td>5</td>
<td>300</td>
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<tr>
<td>Total</td>
<td>1,003,318</td>
<td>265,561</td>
<td>347,568</td>
<td>68,741</td>
<td>71,228</td>
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</table>
Compared to Canada (ca. 29% of animals experience moderate/severe P&D and 14.5% in basic/medical research), US stats very low. Either because US much better at controlling P&D or because P&D is being overlooked. The second option is more likely.
Pain & Distress: Effect on Research

• Unless studying P&D, good science essentially needs P&D controlled/eliminated to limit confounding effects on experiment

• E.G. in clinical medicine, when neonatal anesthesiologists began to use anesthesia/analgesia routinely for neonatal surgery after 1987, infant recovery rates after surgery improved.
  [In mid-1980s, only about 5-10% of neonates received pain relief for major surgery – cf. Anand et al, NEJM, 1987, 317:1321-9]
Examples of P&D Effect on Research

1. Vernon Riley’s studies of stress effects on mouse mammary tumor incidence.

2. The effect of TLC (tender loving care) on athero-sclerosis studies in rabbits.

3. The impact of handling and other treatments on basic metabolite levels.

4. The impact of blood sampling frequencies and volumes on dicoumarol-protein binding studies.
Example 1 – a Riley’s MMTV STUDIES
(V. Riley, 1981, Science 212:1100)

- Stress is a factor in Mouse Mammary Tumor rates caused by MMTV
- Could not use regular lab. housing (in 1970s) because it caused too much stress
- Developed a “low-stress” housing module that reduced noise, light levels and cage changing frequency
- His experimental stressor involved putting cage with mice on a turntable (45 rpm) for 10 minutes.
Example 1 – b
Riley’s MMTV STUDIES
(V. Riley, 1981, Science 212:1100)

• Mice in “low stress” housing had baseline plasma corticosteroid levels of 4-35 ngm/ml of plasma

• Mice in standard housing system had baseline plasma corticosteroid levels of 150-500 ngm/ml of plasma

• [NOTE ALSO: Recent telemetry studies indicate that normal mouse heart rates are 400-450 bpm, not the 650-750 bpm that are measured when mice are handled.]
Example 1 – c
Riley’s MMTV STUDIES
(V. Riley, 1981, Science 212:1100)

The stressor was applied for 10 mins. at beginning of each hour.

The ED50 for tumors was 360 days with stress and 560 days under low stress.
Example 2 - a Rabbit TLC STUDIES
(Nerem et al, 1980, Science 208:1475)

• Group at Ohio State was studying atherosclerosis in rabbits, induced via atherogenic diet

• One day, a trial produced little or no atherosclerotic deposits in the rabbits

• Checked diet – but no change; checked other factors – no discernible change

• A new (female) animal technician had recently taken over care of the rabbits - ????
Example 2 - a 
Rabbit TLC STUDIES 
(Nerem et al, 1980, Science 208:1475)

• On questioning, new technician said care routines had not changed

• However, on observation, it was found that she was handling the rabbits more and brushing their fur for about 20 minutes at a time.

• Ran a trial – some rabbits fed the diet were explicitly not handled, others were handled by the technician as usual
Example 2 - a Rabbit TLC STUDIES  
(Nerem et al, 1980, Science 208:1475)

• The handled rabbits did not develop deposits but the rabbits that were not handled did develop deposits.

• Researchers were still dubious and repeated experiment three times – same result each time.

• Published results in Science but findings have now been confined to the “curiosity” bin of research data because other groups could not repeat them.

• In discussing this with OSU group, they suggested that the “quality” of the TLC was important – i.e. the animal technician had to put heart into TLC.
Example 3 - a Metabolite measures


- Faupel and team were concerned about impact of handling/anesthesia on their liver metabolite levels.

- Were measuring metabolites by standard method of anesthetizing rats, opening up abdomen, “freeze-clamping” liver between aluminum tongs cooled in liquid N2, and then extracting liver metabolites by grinding up frozen liver with frozen perchloracetic acid to look at control of intermediary metabolism.
Developed a rapid “freeze-clamp” method. Rats were acclimated to gentle handling early in morning, were placed on double guillotine to decapitate and open thorax simultaneously, liver would fall out and could be freeze-clamped within 3 seconds. Developed time-course of metabolite level changes by freeze-clamping liver from 3 seconds up to 3 minutes and then looked at impact of not so gentle handling and anesthesia.
Example 3 – c

Metabolite measures

• Found significant anesthetic and handling effects on levels of cellular AMP (x6) (and ADP & ATP), Glucose-6-phosphate (x2) and other important regulatory metabolites.

• Concluded that standard methods gave erroneous results. Others have subsequently shown similar effects (e.g. normal heart rates in rats and mice much lower than usually measured, blood parameters affected by handling, and of course various hormones and brain metabolites affected by handling and anesthetics/analgesics).

• However, can find recent publications measuring metabolites that use techniques that have been shown by several authors to produce confounding results. The “proper” approach requires a lot of time and effort.
Example 4-a: Effects of Sampling Vol. & Freq.

Study of Dicoumarol-Protein Binding in Blood in the Rat

<table>
<thead>
<tr>
<th></th>
<th>2ml every 2 hrs for 12 hrs</th>
<th>1ml every 2 hrs for 12 hrs</th>
<th>0.5ml every 2 hrs for 12 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit - % reduction</td>
<td>47</td>
<td>27</td>
<td>12.5</td>
</tr>
<tr>
<td>Plasma Alb. - % reduction</td>
<td>31</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Free Dicoum. Fraction (%)</td>
<td>5.1</td>
<td>1.3</td>
<td>0.6</td>
</tr>
<tr>
<td>FFA % Incr.</td>
<td>240</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>
Example 4-b: Effects of Sampling Vol. & Freq.

- The sampling frequency clearly has a dramatic effect on blood parameters.

- Free Fatty Acid levels are an index of stress – FFA increase because of release of catecholamines – and it is clear that 2ml/hr is stressful.

- However, 1ml/hr, which does not increase FFA significantly, has an impact on other measures.
• These four examples are somewhat dated but are classic illustrations of the principle – any relatively quick review of the literature today will produce a similar set.

• They illustrate that relatively subtle stressors can have a significant effect on experimental data.

• They also illustrate that, although difficult, it is possible to devise measures that assess animal stress.
• Thus – the development of a variety of stress measures and their application in the laboratory should have two beneficial consequences:
  – Improving the quality of one’s data
  – Reducing the levels of distress experienced by laboratory animals

• This should please granting agencies, academic departments and the public!