Example of refinement in animal experiments: the case of levodopa-induced dyskinesia

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Refinement in 3Rs

Refinement refers to

methods that minimise the pain, suffering, distress or lasting harm that may be experienced by research animals, and which improve their welfare.

Refinement applies to all aspects of animal use, from their housing and husbandry to the scientific procedures performed on them.

In our present case, refinement lead immediately to the other R, namely **reduction**

Levodopa-induced dyskinesias (LIDs)

- No treatment modifying the progression of Parkinson's disease is available

- Levodopa treatment is effective, but often complicated by dyskinesias
  - Abnormal involuntary movements of the head, trunk and/or limbs
  - Prevalence:
    - 40 % of patients after 4 to 6 years of treatment
    - 90 % after 9+ years
  - Can lead to disability and a significant loss of quality of life

Levodopa-Induced Dyskinesias (LID) in Parkinsonian patients

Types of LIDs:

Peak dose dyskinesias
Diphasic dyskinesias
Off-period dyskinesias

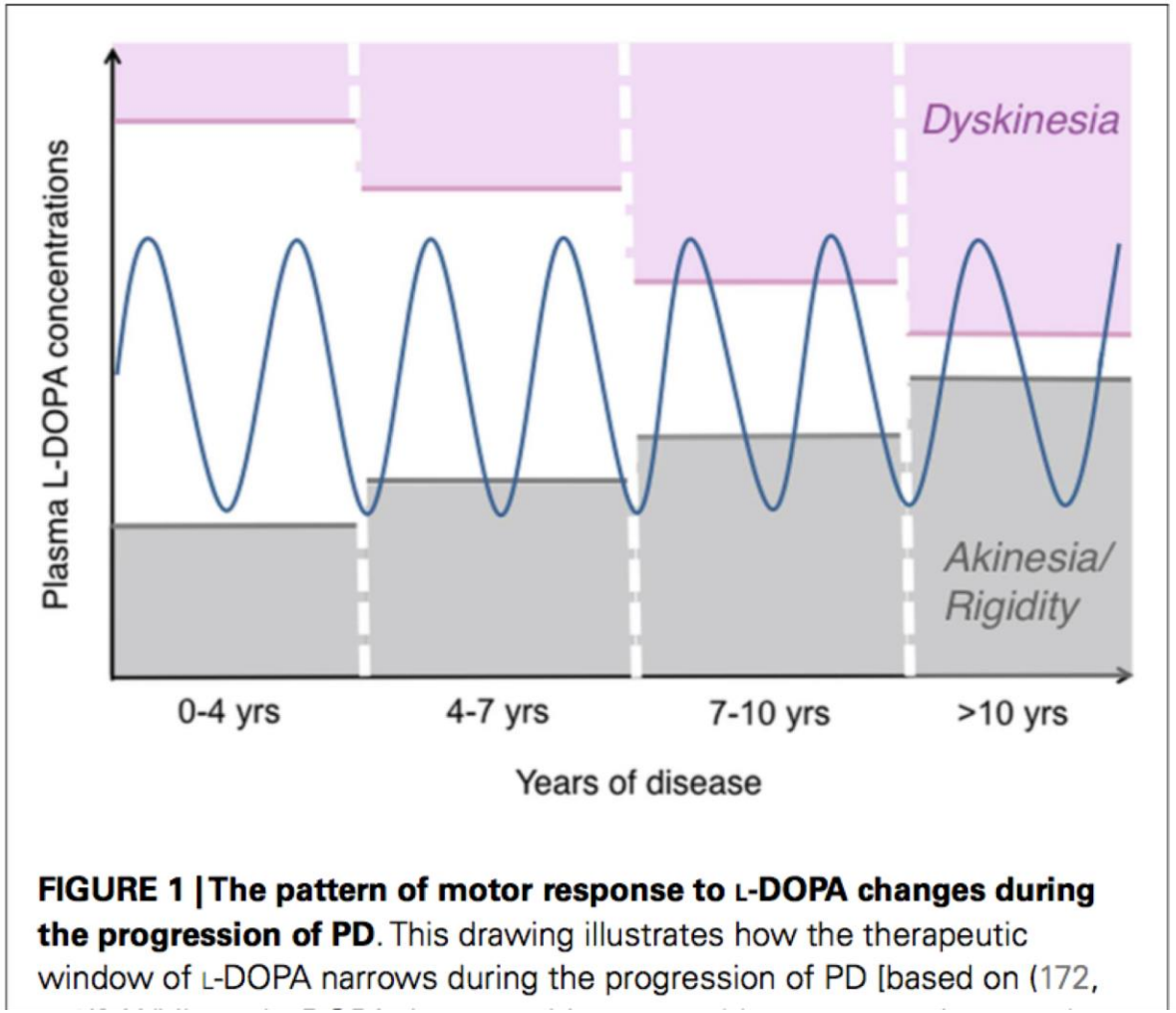

FIGURE 1 | The pattern of motor response to L-DOPA changes during the progression of PD. This drawing illustrates how the therapeutic window of L-DOPA narrows during the progression of PD [based on (172,

https://www.youtube.com/watch?v=4S56JGo826g

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Levodopa-induced dyskinesias (LIDs)

- Treatment options for LIDs are sparse and with significant drawbacks
  - Delaying levodopa treatment
  - Amantadine: limited effect
  - Deep brain stimulation (DBS) surgery: promising but invasive

- Molecular mechanisms of LIDs are largely unknown
  - Supersensitive signalling downstream of D1 receptors?
  - Involvement of serotonergic system: LIDs are reduced by serotonergic denervation of the raphe nuclei as well as serotonergic antagonists
  - Other systems under investigation: glutamate, GABA, adenosine, cannabinoid, noradrenaline etc.

Mouse model of levodopa-induced dyskinesia (LID) from the literature

- Mice with unilateral 6-OHDA lesion
  - Striatal or medial forebrain bundle (MFB) injection(s)
  - Typical 6-OHDA dosage:
    - MFB: 3 µg, Striatum: 2 x 6 µg
  - Choice of injection site and dose: high mortality vs. lesion severity vs. low incidence of LID

- Chronic levodopa+benserazide treatment (i.p. or s.c.)
  - Typical daily levodopa dosage:
    - MFB lesions: 3–6 mg/kg
    - Striatal lesions: 10–30 mg/kg
  - Typical benserazide dosage: 12–15 mg/kg

- Rating of dyskinesias with abnormal involuntary movement (AIM) rating scale

Comparing striatal STR ja MFB lesions

STR lesion	MFB lesion
2x6 microg 6-OHDA injections	1x 3 microg injections
Low mortality rate	Higher mortality rate, requires extensive post operation care
Higher levodopa doses needed	Lower levodopa doses are enough
Axial dyskinesias, rotation	All types of dyskinesias: Rotation Axial dyskinesias Forelimb dyskinesias Orolingual dyskinesias

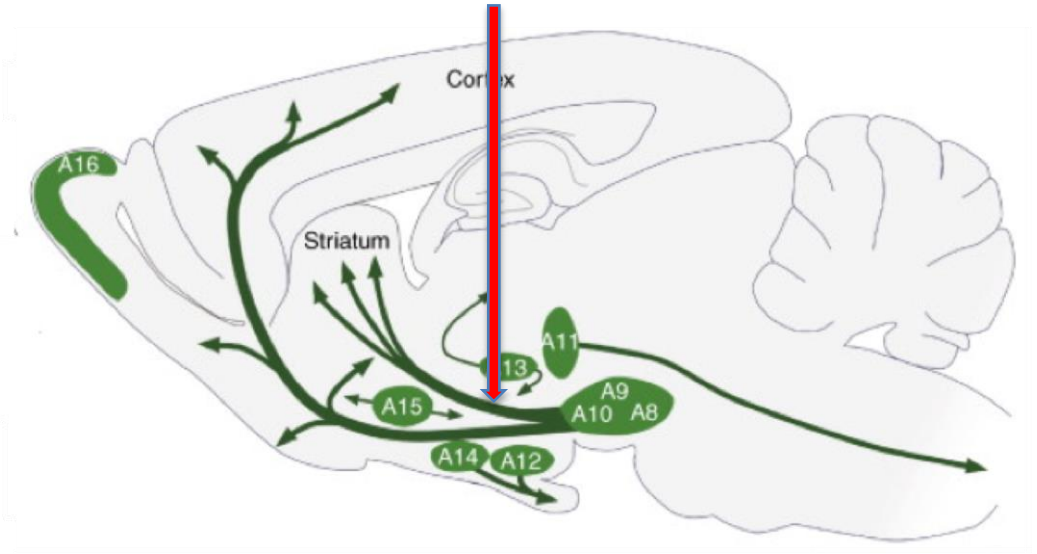
Abnormal involuntary movement (AIM) rating scale

- Validated scale for rats and mice[1,2,3]
- AIM definition: primarily contralateral, purposeless and repetitive abnormal involuntary movements disturbing or replacing normal motor behavior
- 4 types of AIMs:
  - Locomotive: circular locomotion with contralateral side bias
  - **Axial:** lateral deviation of head, neck and trunk towards contralateral side
  - **Limb:** Movements of contralateral forelimb
  - **Orolingual**: Bilateral jaw movements, grimacing, tongue protrusion; biting of contralateral limb
- Severity rating from 0 to 4 (absent – – – continuous and non-interruptable)
- 1 min monitoring periods at 30 min intervals, continued (for example) for 2,5 h after levodopa injection
- Ratings from different types and time points can be summed to obtain ”integrated rating”

1 Winkler et al. 2002, Neurobiol Dis 10
2 Lundblad et al. 2004, Neurobiol Dis 16
3 Lundblad et al. 2005, Exp Neurol 194

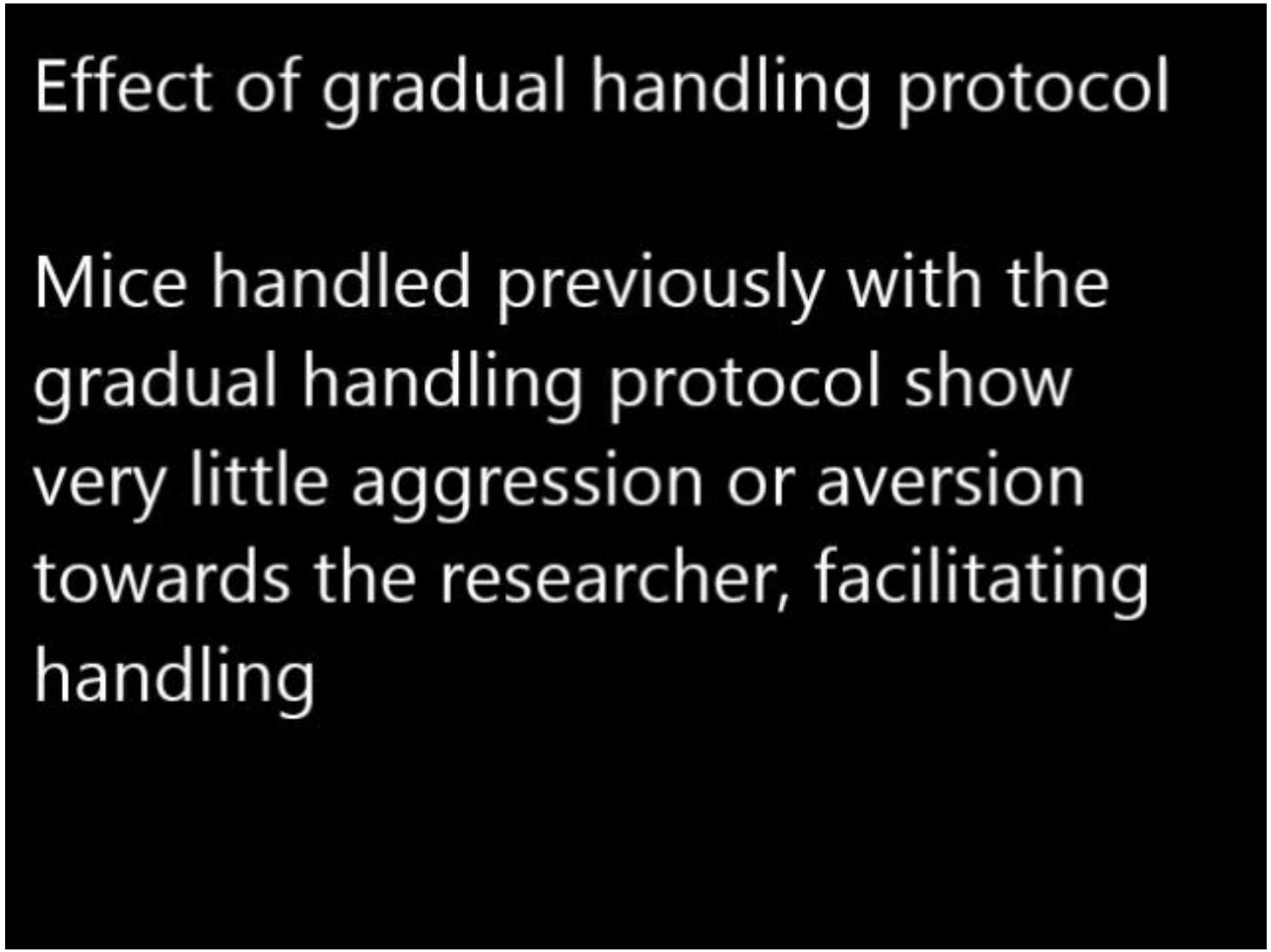
6-OHDA lesion in medial forebrain bundle (MFB)

- 6-OHDA (3 µg) is injected into the right **MFB =A/P -1.2, D/M -1.1, D/V -5.0**



- Injection volume 0.2 microl at a rate 0.1 microl/min
- Needle kept in its place for 5 min, then slowly withdrawn within 2 min

Taming the animals: ensuring the well-being of animals (and the researcher) throughout extensive experiment series



High mortality after MFB lesions: Extensive postoperative care is critical!

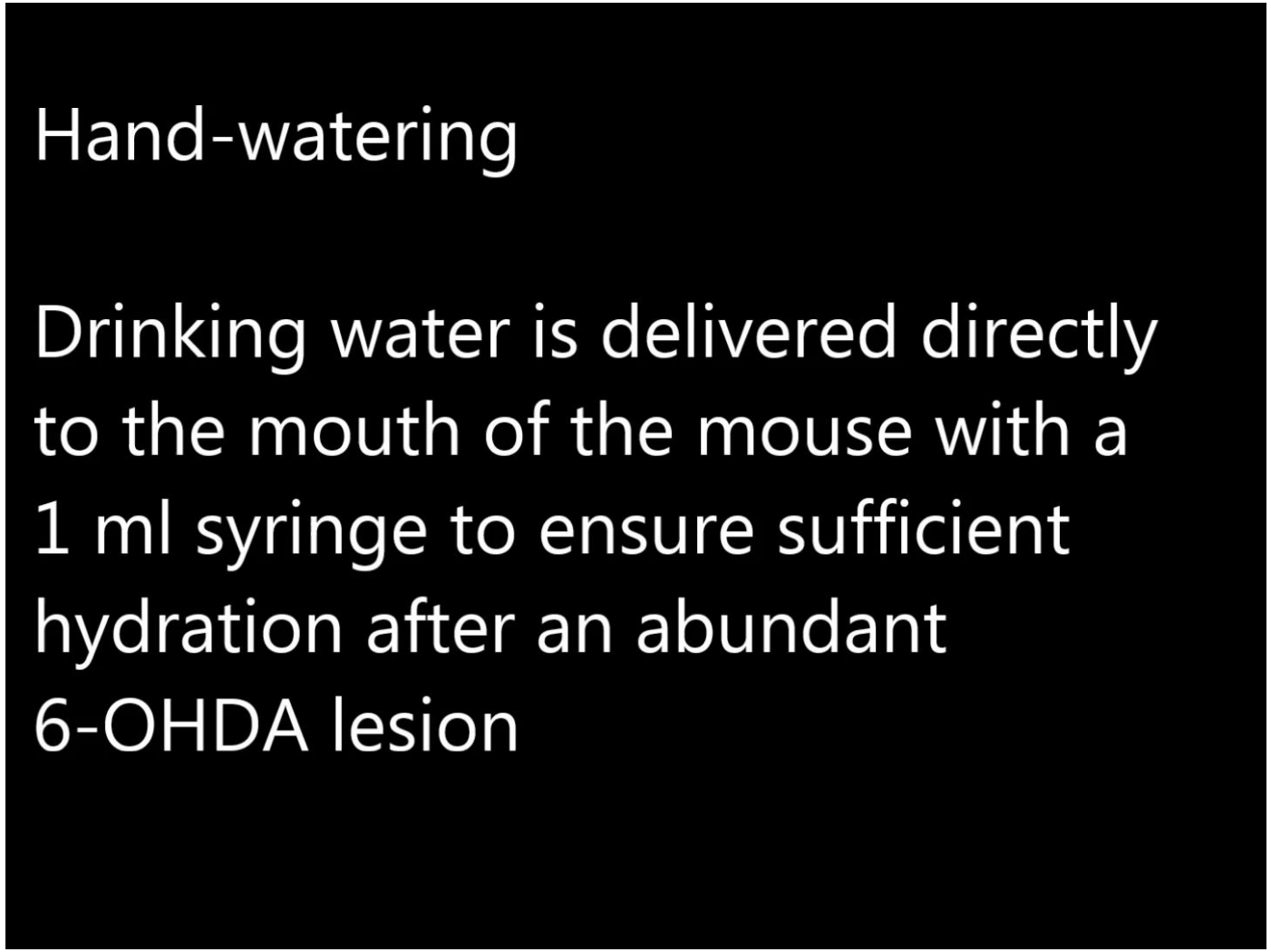
- Females used mostly
- Group housing necessary! Healthy mouse placed in the cage to take care of the operated ones that were in bad condition.
- Houses and warm nesting material introduced prior to lesion to prevent hypothermia

- Warm saline injections after surgery: 1 ml s.c. 1-2 x day for 10 days
- First days pain relief (buprenorfine hydrochloride and later carprofen)
- High energy diet (Bacon Softies™) and gel (DietGel Recovery)
- Also additional food (Nutri-plus gel)
- Food placed in bottom of the cage within reach
- Also hand-feeding, when necessary
- Penis prolapse treated with antibiotic creme (use females if possible!)

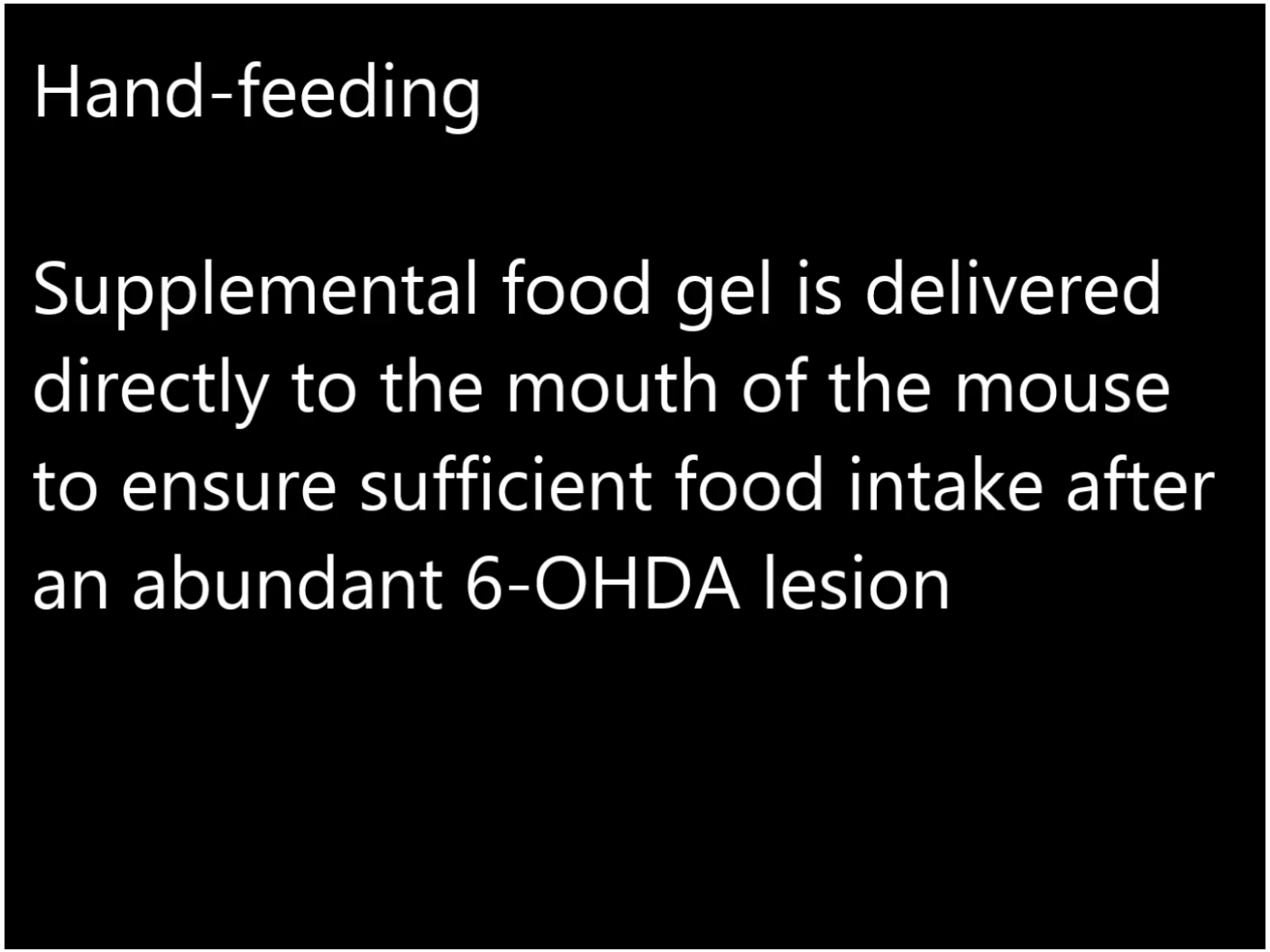
-> mortality now only 15-20%

Severe weight loss could not be prevented (20-25 %) but weight was back to normal in 3 weeks.

You can easily give drinking water by hand to the tamed mouse



Tamed mouse can be also hand-feed after surgery



Detailed information here:

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Original scientific article

Implementation of improved postoperative care decreases the mortality rate of operated mice after an abundant 6-hydroxydopamine lesion of nigrostriatal dopaminergic neurons

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Summary

- Medial forebrain bundle 6-OHDA injection produces a severe lesion in DAergic neuronal pathways modeling advanced state of parkinsonism
- MFB 6-OHDA lesioning was necessary to induce all types of levodopa-induced dyskinesia in the mouse model
- Preoperative taming protocol and extensive postoperative care protocol reduced the mortality after the MFB lesioning
- The refinement of the procedure lead to increased animal (and researcher) well-being and also reduced the overall number of animals needed in the experiments, while ensuring the high quality research data

Thank you!

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