Multi-laboratory study design

Breeding Facility
time-mated pregnant females

- **90 time-mated pregnant females C57BL/6JRj** from the same breeding stock of a commercial breeder (Janvier Labs, France) were randomly **allocated to 5 different** rearing laboratories (n = 18 per lab).

Rearing Laboratories

both male and female offspring were reared until the age of 8 weeks

- **At weaning (PND 22),** in each rearing laboratory **up to 12 litters with at least 3 pups of each sex were selected** randomly from all litters. To achieve n=12, these were complemented by litters with at least 2 pups of each sex.
- From each litter 3 (or 2) **offspring** per sex **were reared until the age of 8 weeks (PND 56)** according to the specific protocols of housing and husbandry of each of the 5 animal facilities.
- **At PND 57 one male and one female of** all cages with 3 mice were sacrificed to control for changes induced by the transport to, and housing in, the test laboratory.

The remaining pairs of male and female offspring (n = 240) were transported from the 5 rearing facilities to the testing facility at the University of Bern.

Testing Facility

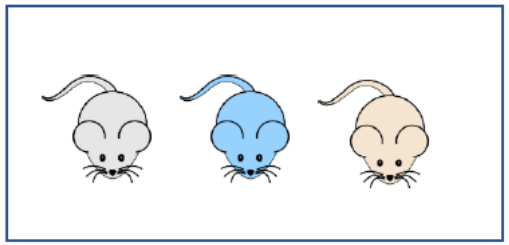
measures of anxiety stress response

- After an acclimation period of 2.5 weeks, **mice were tested for phenotypic differences** in HPA stress reactivity (primary outcome variable) and anxiety-related behaviour (secondary outcome measures).
- For this, **120 mice were used** (one mouse per sex per litter), while their cagemates (n = 120) remained test-naïve. Molecular analyses are going to be performed on test-naïve animals to avoid the effect of behavioral testing on gene expression and epigenetic profile.

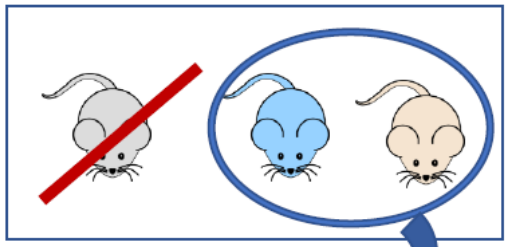
All mice (n=240) were sacrificed a week after the last test (PND 100; ~14.5 weeks of age).

Multi-laboratory study design

Each rearing lab: 3 mice per cage per sex
from weaning (PND 22) until 8 weeks of age (PND 56)



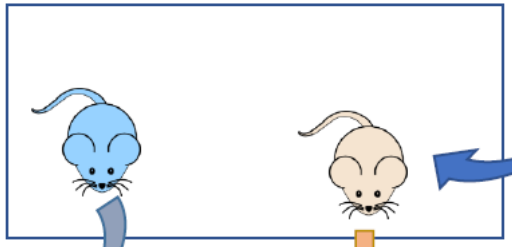
PND 56 (8 weeks of age)



Cage mates are transported from each rearing facility to Bern testing facility.
All animals are transported from each rearing lab in the same vehicle.
Even animals from Bern rearing lab had a ride to Zurich and back ☺ in order to provide the same conditiones for every single mouse.
Cage mates are treansported together in the special transport boxes.

Sacreficed at PND 56,
within rearing facility

Transported to Bern
(testing facility)



Underwent
behavioral testing for anxiety
and HPA reactivity and
sacreficed at 14 weeks of age

PND 98 (14 weeks of age)

test-naïve mouse, sacrificed at 14 weeks of age
for epigenetic and gene expression analyses and
microbiota profiling

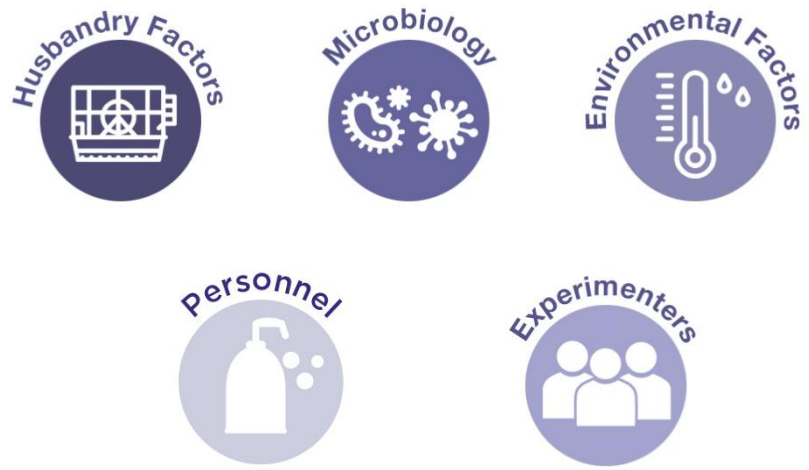
PND 98 (14 weeks of age)

Multi-laboratory study design

Factors that were laboratory specific

All factors related to housing and husbandry conditions

- Type of cages, amount and type of bedding, nesting and enrichment material
- Mixed-strain or same-strain housing room condition
- Single-sex or mixed-sex housing room condition
- Lighting conditions (Including day-night rhythm)
- Frequency and method of mice handling, daily checks, weighing
- Cage and food changing frequency
- Type of food
- Temperature, humidity and background noise…



Factors that were standardized between different rearing laboratories

- Upon arrival, pregnant dams were housed individually
- Dams were monitored daily for parturition (but time of day is lab-specific)
- **Day of birth was defined as postnatal day 0 (PND 0)**
- **Litters were not culled during the lactation period**
- Pups were not individually identified during the lactation period.
- Pups were weaned on PND 22.
- There was no permanent marking at weaning.
- **After weaning, all mice were housed in in same-sex groups of 2 or 3 littermates**