**Burrowing Protocol**

[**1.** **Study design (following Good Laboratory Practice guidelines is encouraged)** 3](#_Toc433297892)

[**2.** **Equipment** 4](#_Toc433297893)

[**3.** **Animals** 4](#_Toc433297894)

[**4.** **Training** 5](#_Toc433297895)

[**5.** **Testing** 6](#_Toc433297896)

[**6.** **Model details** 6](#_Toc433297897)

****

# **Study design (following Good Laboratory Practice guidelines is encouraged)**

|  |  |
| --- | --- |
| Sample size calculation | Pilot burrowing data from 3 days following induction of inflammation (n=6 and n=5 for control and CFA group, respectively). Parameters were difference in means=951.79 g, SD=549.29 g, α=0.05 and power=0.8. An adequately powered study required an n=7 per group and n=8 when testing two and three groups, respectively (SigmaPlot 11.0 [ANOVA]) |
| Randomisation | Follow local procedures; randomisation based on baseline performance to ensure each group has a comparable baseline in terms of mean value and variation  |
| Allocation concealment | Follow local procedures |  |
| Blinding | Follow local procedures |  |
| Code breaking | At the end of the study |  |
| Exclusion criteria | Animals burrowing less than 2 standard deviations of total mean at any of the baseline sessions**; Amendment**: Or animals burrowing less than 500g at any of the baselines; **Amendment**: No animals should be excluded based on burrowing behaviour to allow for IIT and PP analysisAll exclusions have to be reported |

# **Equipment**

|  |  |  |
| --- | --- | --- |
| **Burrowing Material** |  |  |
| Type | Gravel |  |
| Size | 5-10 mm (pea shingle) – dependent on availability |
| Washing procedure | Water; if possible autoclave at the end of study |
| Amount per tube | 2.5 kg |  |
| **Burrowing Tube** |
| Dimensions | 320 mm (length) x 100 mm (diameter) |
| Raised form ground | 60 mm at open end |  |
| Material | Plastic |  |
| Washing procedure | Water; if possible autoclave at the end of study |
| **Home/Test Cage** |  |  |
| Size | Standard home cage fro group housing (about 595x380x200 mm) – dependent on local variation |
| Washing procedure | New cage after each testing day; wipe clean in between sessions |
| Bedding | Paper towel/absorbent sheet - optional |

# **Animals**

|  |  |  |
| --- | --- | --- |
| Species | Rat |  |
| Strain | Sprague Dawley (preferred strain, although Wistar is also acceptable) |
| Gender | Male |  |
| Supplier | Dependent on supplier used locally  |
| Weight at start of training | 250-350 g (adult) |  |
| Acclimatisation before start of experiment | 7 days (local variations allowed) |
| Housing | Group housed; temperature and humidity controlled environment; environmental enrichment in cage |

# **Training**

|  |  |  |
| --- | --- | --- |
| **Conditions** |  |  |
| Time of day | During light phase (preferred from 7 am-1 pm) |
| Lighting | No specification given (suggested to use low lighting) |
| Noise | No specification given |  |
| Test room | Separate from housing room |  |
| Food/water availability | No |  |
| **Protocol** |  |  |
| Acclimatisation | 30 min in testing room (in home cage); followed by 30 min in empty test cage before filled burrowing tube is added |
| Duration of burrowing | 1 hr |  |
| Number of animal per test cage | 2 |  |
| Number of habituation sessions | 0 |  |
| Number of training sessions | 2 (consecutive days) – dependent on performance; might need more |
| Social facilitation | If a pair did not burrow during the first session, one of the rats is swapped with a rat from a good burrowing pair – rats will undergo a second training session even if they are not required for social facilitation  |

# **Testing**

|  |  |  |
| --- | --- | --- |
| **Conditions** |  |  |
| Time of day | During light phase (preferred from 7 am-1 pm) |
| Lighting | No specification given (suggested to use low lighting) |
| Noise | No specification given |  |
| Test room | Separate from housing room |  |
| Food/water availability | No |  |
| **Protocol** |  |  |
| Acclimatisation | 30 min in testing room (in home cage); followed by 30 min in empty test cage before filled burrowing tube is added |
| Duration of burrowing | 1 hr |  |
| Number of animal per test cage | 1 |  |
| Number of habituation sessions | 0 |  |
| Number of baseline sessions | 2  |
| Number of testing sessions | 5 (days 1, 2, 3, 7 and 10 following model induction) |
|  |  |
| Burrowing tube/testing cage assignment | No burrowing tube assignment, but each rat is assigned to a test cage position on the cage rack, which remains during the study – dependent on local set-up |

# **Model details**

#

|  |  |
| --- | --- |
| CFA | Sigma-Aldrich (F5881) – dependent on availability |
| Volume | 100 μl |  |
| Concentration | 1 mg/ml = 100μg |  |
| Sham | Same volume of saline |  |
| Anaesthesia | Short isofluorane |  |