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Animal welfare assessment after severe traumatic brain injury in rats

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Abstract

Severe traumatic brain injury (TBI) is a multifactorial injury process involving respiratory, cardiovascular, and immune functions in addition to the brain. Thus, live animal models are needed to study the molecular, cellular, and systemic mechanisms of TBI. The ethical use of laboratory animals requires that the benefits of approaches be carefully weighed against potential harm to animals. Welfare assessments adapted to severe TBI research are lacking. Here, we introduce a scoresheet to describe and monitor potential distress in animals, which includes general welfare (body weight, general appearance, and spontaneous behavior) and TBI-specific (respiratory function, pain, locomotor impairment, and wound healing) indices. Implementation of this scoresheet in Sprague-Dawley rats subjected to severe lateral fluid percussion (LFP) TBI revealed a period of suffering limited to four days, followed by a recovery to normal welfare scores within 10–15 days, with females showing a worse impact than males. The scores indicate that animal suffering in this model is transitory compared to TBI consequences in humans. The scoresheet allows for the implementation of refinement measures including (1) analgesia during the initial period following TBI and (2) humane endpoints set (30% weight loss, score ≥ 90 , and/or respiratory problems). This animal scoresheet tailored to TBI research provides a basis for further refinement of animal research paradigms aimed at understanding or treating the sequelae of severe TBI.

Keywords: Traumatic brain injury, rat welfare scale, humane endpoint.

Introduction

Traumatic brain injury (TBI) is a leading cause of death and disability worldwide. In humans, the Glasgow Coma Scale is used to classify patients' state of consciousness and TBI severity, ranging from mild to moderate and severe. Among the 69 million people affected by TBI each year worldwide, an estimated 5.48 million suffer from severe TBI.¹ Such patients suffer from complex and multifactorial injuries and they have a high mortality rate.² Therefore, modelling this pathology in biomedical research is a priority.

Primary injuries are apparent immediately after a TBI has been sustained, and then evolve over hours, weeks, or even years.^{3, 4} In addition to brain changes, TBI can also affect respiratory, cardiovascular, and immune functions.⁵ Consequently, TBI is difficult to model in vitro, in cell cultures, or in brain explants, and in vivo animal research is warranted.⁶ The ethical use of laboratory animals requires that the benefits of the approach be carefully weighed against potential harm to animals. In TBI research, ethical concerns are especially relevant to severe TBI, after which animals may experience suffering. Animal research laboratories worldwide have committed to the three Rs—replacement, reduction, and refinement⁷—and they are additionally required to abide by national regulations.^{8, 9} Moreover, animal welfare can impact on scientific outcomes in terms of reliability and reproducibility.¹⁰ Thus, for both ethical and scientific reasons, it is important to assess animal welfare after TBI accurately. The first attempts to understand and evaluate general animal welfare in research laboratories were developed in the 1980s based on behavioral and physiological criteria, such as body weight, cardio-respiratory function, and general appearance.^{11, 12} Since then, scoresheets have become increasingly specific to species and the pathology being modeled. Severity scoring rubrics have been developed for use in welfare assessment and to define humane endpoints in toxicology or cancer research. Although a brain injury-specific scoresheet has been proposed,¹³ specific scoring grids adapted to TBI research are lacking.

Here, we introduce a scoresheet designed to monitor animal welfare following TBI that includes both general welfare indices and TBI-associated characteristics. This scoresheet was used to describe animal welfare for a period of 160 days after severe TBI induced by the lateral fluid percussion (LFP) method (5-6 months and up to 1 year is considered a long-term timeframe for TBI consequences in rodents).¹⁴ Following this descriptive approach, observations documented via the scoresheet provided a basis for the definition of humane endpoints, and refinement of animal care after severe TBI.

Materials and methods

Animals

Experimental protocols were approved by the CELYNE (CEA-042) committee on animals in research. APAFIS#10019-201704211705870), according to European Directive 2010/63/UE and abode to the ARRIVE guidelines. Forty-two female and male adult (10-week-old) Sprague-Dawley rats (Envigo, Gannat, France) were randomized into four groups: 10 sham females; 9 sham males; 11 TBI females; and 12 TBI males (the TBI group was designed to be larger than the sham group because of possible deaths that could be expected following severe TBI). There was no significant difference in body weight between the sham and TBI groups (females 219 [212.5–223.5] g, males 264.0 [251.5–279.5] g). The rats were housed in pairs, a sham and TBI rats, on a 12-h light/dark cycle with controlled temperature ($23 \pm 3^\circ\text{C}$) and hygrometry ($50 \pm 5\%$). Enrichment included one wooden stick and a sizzle nest. Rats had *ad libitum* food and water access. After one week of acclimatization, the animals were handled gently for 5 min/day for one week to habituate them to the laboratory conditions and the experimenter.

Surgery and TBI induction

TBI was induced using the LFP model.^{15, 16} Rats were anesthetized with isoflurane (induction, 4.5%; maintenance, 1.8-2% in 30% oxygen/70% air) and received a subcutaneous infiltration of buprenorphine (0.02 mg/kg, Buprecare[®], Axience, Pantin, France) and glycopyrronium bromide (0.1 mg/kg, Robinul-V[®], Centravet, Lapalisse, France). After 25 min, Animals were intubated with an endotracheal tube coated externally with pramocaine (Thronothane[®], Biocodex, Gentilly, France) and anesthesia was maintained via the artificial ventilation system (Ispra MA1 55-7058, Harvard Apparatus^R, Edenbridge, UK; tidal volume = 6 ml/kg, breath rate = 60/min, positive end expiratory pressure = 5 cm H₂O). Animals received an additional subcutaneous injection of ropivacaine (3.75 mg/mL, Naropeine, AstraZeneca LP, Courbevoie, France) and lidocaine (5 mg/mL, Xylovet[®], Ceva, Libourne, France) in the scalp 10 min prior to incision. A circular craniotomy (4 mm diameter) centered 3.8 mm caudal to Bregma and 3.00 mm lateral to the midline was performed over the right hemisphere. A luer-lock syringe tip was attached to the skull and over the cranial window with cyanoacrylate and dental acrylic cement. Tubing (100 cm in length and 2.5 mm inner diameter, model TU0425, SMC) filled with saline was connected on one side to the LFP device (Custom Design and Fabrication, Virginia Commonwealth University, Richmond, VA), and on the other side, to the luer-lock tip cemented to the cranial window. Anesthesia was stopped and TBI was induced upon recovery of the hindpaw withdrawal reflex. This lighter anesthesia corresponded to the transition from plane 2 to plane 1 of stage 3 (moderate) anesthesia as defined by Siddiqui and Kim (2021),¹⁷ and was shown to limit the loss animals by respiratory arrest after TBI. The pendulum was released, and stroke the water column, inducing a single 20 ms–5 atm fluid percussion pulse. The pressure of the shockwave usually decreased in intensity when it reached the dura mater, and was also measured at that location using an MLT0699 Disposable Blood Pressure Transducer (AD Instruments, Oxford, UK). A few drops of doxapram hydrochloride (Dopram-V[®], Centravet) were applied on the tongue before injury, and mechanical ventilation was

resumed without anesthesia. The scalp was sutured and the rat received 2 mL of saline in the flank subcutaneously. The same procedure, including a scalp incision and cranial window, was used for the sham group, but without TBI induction. Body temperature was set at 37 °C with a temperature-controlled heating pad (LSI Letica, Barcelone, Spain). Breathing and heart rate were monitored with a MouseOx (STARR Life Sciences Corp., Oakmont, PA, USA) or Etisense system (Etisense, Lyon, France). After surgery, animal consciousness was assessed with the Tübingen-Boston Rat Coma Scale (RCS).¹⁸ The RCS was completed every five minutes for a total score ranging from 0 (coma) to 16 (normal full consciousness).

Rats were injected with buprenorphine at 8h and 16h after surgery, and during the first 24h they were heated with a red lamp. Moistened food was given for 1-2 weeks to facilitate food intake.

Post-TBI welfare scoresheet

The 11-item welfare scoresheet (Figure 1) was completed daily (8:00 to 9:00 a.m., 3 min by cage) for the first 28 days post-TBI by three experimenters, then by one, until 160 days. The experimenters were one PhD student with six years of laboratory experience with rodents, a zootechnician with 10 years' experience, and a master's degree student with one year of experience. The three scorers examined the animals independently and were alone in the room during scoring. Blinding was not possible because the difference in animal behavior after TBI was obvious. Animals were first observed remotely through transparent cages to determine their general condition, grimace score, breathing rate, and spontaneous behavior. The experimenter then opened the cage and scored each animal's reaction to handling.

General condition, physical appearance included coat (presence of piloerection, unkempt fur), eyes (presence of porphyrin), forelimbs and nose (clean or stained). We should note that adult animals have a tendency for a rougher, less silky coat than adolescent animals, and this should be differentiated from suffering.

Grimace scale: pain and discomfort were indexed based on facial expressions with the Rat Grimace Scale developed by Sotocinal et al.¹⁹; rated expressions include orbital tightening, nose/cheek flattening, and ear and whisker changes.

Breathing rate was observed visually, with attention to potential breathing problems (open mouth, wheezing, vocalizations or jerking) and cyanosis (paleness or blue coloration of the limbs, tail, ears, or nose).

Spontaneous behavior: animals must be housed in pairs to enable observation of social behavior and note any isolation from the congener. Subsequently, the experimenter moved each cage to a bench and opened it while watching the animal's behavior.

Reaction to handling: prostration, isolation, absence of movement or escape from the experimenter were considered signs of poor welfare.

Locomotion/gait were scored while the animal was on a table, with specific attention to the signs of atonia, loss of balance, and/or weakness.

Interaction with environment, enrichment: evidence of interaction with enrichment items, such as nibbling of the wooden stick or nesting.

Injury or sequelae: the surgical scar and incisor growth were examined daily. Because malocclusion can reduce food intake and lead to weight loss, incisor filing was performed after isoflurane induction (4.5%, 2-3 min procedure) with miniature saw (Dremel, France) whenever necessary until resumption of normal wear.

Hydration: hydration was determined by pinching the upper back skin and measuring its relaxation time.²⁰

Body condition and weight: each animal was observed visually and palpated on its back and flanks to feel the prominence of its bones.²¹

Four score-based welfare stages were defined: 0–10, good physical condition and absence of pain; 11–60, possible discomfort or pain, triggering increased monitoring and adapted

corrective measures, including subcutaneous 50-mg glucose injection and/or appetitive food 3-4 times/day (if weight loss > 5% or dehydration), buprenorphine injection (0.02 mg/kg, subcutaneous) for pain or injury; 61–89, substantive pain and probable general suffering, triggering veterinarian advice; and ≥ 90 , humane endpoint triggering euthanasia. Grades assigned on the scoresheet increased exponentially as the symptoms worsened, following a similar approach to Pinkernell et al.¹³

Pinkernell et al.¹³ proposed a scoresheet for both TBI and subarachnoid hemorrhage models, that was used here to evaluate animal welfare independently of our own scoresheet for comparison during 20 days.

[Insert Figure 1]

Endpoints

Two peri-operative endpoints were defined: dural tear; and respiratory failure requiring mechanical ventilation for >45 min. Subsequently, a veterinarian indicated if euthanasia was warranted based on daily interaction. When necessary and at the end of the experiments, euthanasia was performed with 5% isoflurane anesthesia followed by lethal intraperitoneal injection of barbiturates (100 mg/kg, Euthasol[®], Centravet). Finally, the brain was removed for further histological analysis.

Data analysis

Data are expressed as median [interquartile range]. Data were analyzed in Prism[®] version 8.4.3 (Graphpad software LLC, San Diego, CA, USA) or R software 4.0.3.²² Raw data were first analyzed for normality with the Shapiro-Wilk test. For two-group comparisons, normally and non-normally distributed values were analyzed with Student's unpaired t-tests and Mann-

Whitney U tests, respectively. Including both male and female animals in the study warranted statistical analysis with a two-way ANOVA for sex and TBI. Before the two-way ANOVA was performed, data samples were tested for homoscedasticity with the Levene test and normality of residues. For non-normally distributed residues or heterogeneity, raw data were transformed with the Box-Cox power transformation.²³ If the two-way ANOVA revealed an interaction between sex and TBI, a Mann-Whitney U post-hoc test adjusted by the Bonferroni method was applied to identify specific differences. If not, no post-hoc tests were performed. Differences were considered significant at $p < 0.05$ for ANOVAs and at $p < 0.025$ for repeated Mann-Whitney U tests (two repetitions for males and females).

Inter-experimenter variability was studied by calculating intraclass correlation coefficient, expressed with 95% confidence intervals based on a mean-rating ($k = 3$) absolute-agreement/two-way mixed-effects model, and interpreted based on that of Koo et al.²⁴

Results

Severe TBI induction and acute recovery

Although LFP induction produced a similar 5 atm shockwave in the water column, dural peak pressure diminished to a lower value in males (2.5 [2.4–2.6] atm) than in females (2.8 [2.6–2.8] atm; $t(21) = 3.04$; $p = 0.006$). Eight rats placed in the TBI group were not included in the analysis: five were euthanized due to a dural tear; one died spontaneously in the first hour following TBI; and two did not show evidence of TBI from RCS.

Relative to sham animals ($N = 19$; 10 females), the remaining TBI animals ($N = 15$; 8 females) required an extended surgery-recovery time, as indicated by hindpaw withdrawal reflex (36.0 [18.0–72.0] min vs. 0 min in sham animals), righting reflex recovery (84.0 [51.0–90.0] min vs. 3.0 [0.0–30.0]; $F(1,30) = 134.40$; $p < 0.001$) and RCS normalization (100.0 [75.0–115.0] vs.

5.0 [5.0–10.0] min; $F(1,30) = 161.4$; $p < 0.001$) (Figure 2). Immediately after TBI, we observed a 10 to 30-s respiratory arrest and abnormal heart rhythms (tachycardia and bradycardia) with cyanosis for about 5 min.

Female rats took more time to recover their righting reflex (100.0 [82.5–145.0] min) than males (85.0 [40.0–150.0]; $F(1,30) = 7.44$; $p = 0.011$), and showed a trend toward a longer hindpaw withdrawal reflex recovery (females, 57.0 [26.25–73.50] min; males, 34.0 [10.0–46.0] min; $t(13) = 1.91$; $p = 0.088$). Finally, 11/15 TBI rats (4 females and 7 males) had a subdural hematoma. These observations indicated severe TBI.

[Insert Figure 2]

Applying our scoresheet to rats with severe TBI

Score of TBI animals worsened dramatically during the first 1–2 days postoperatively and then improved gradually thereafter until reaching stable basal values (< 0.001 ; post-hoc Mann-Whitney U sham vs. TBI female $p < 0.001$) with an interaction of sex and TBI variables ($F(1, 30) = 7.19$; $p = 0.02$) but no significant difference between female and male groups ($F(1, 30) = 1.577$) (Figure 3(b)).

Welfare worsening following TBI was attributable to virtually all scoresheet items. Compared to sham animals, TBI animals took longer to recover a silky coat (female, $p < 0.001$; male, $p = 0.004$) and had more days with probable pain (grimace scale ≥ 10 ; F, $p < 0.001$ and M, $p = 0.008$), dyspnea (F, $p < 0.001$ and M, $p = 0.012$), abnormal responses to handling (F, $p < 0.001$ and M, $p = 0.002$), and impaired locomotion (both $p < 0.001$). TBI rats had greater weight loss than sham rats (F, $p < 0.001$ and M, $p = 0.005$), and took several days to normalize (female 16 days, $p < 0.001$ and male 8 days, $p = 0.016$) (Figure 3 (c)). Finally, dehydration paralleled the

changes observed in body weight, lasting 8–16 days in TBI rats, versus 0–1 day in sham animals (F, $p < 0.001$ and M, $p < 0.005$). Detailed results for each scoresheet item are shown in Table 1.

TBI female rats took longer than males to recover a normal coat (10.5 [7.0–26.8] vs. 4.0 [3.0–8.0] days, $p = 0.019$), were dehydrated longer (15.5 [9.0–23.3] vs. 7.0 [4.0–8.0] days, $p = 0.007$), and took longer to recover their normal body condition (12.5 [9.0–23.5] vs. 6.0 [3.0–9.0] days, $p = 0.01$). Body condition (thinness, 12.5 [9.0–23.5] days) and interaction with the environment (1.0 [0.3–2.8] day) were significantly impacted compared to sham animals in females only ($p < 0.001$ and $p = 0.002$, respectively) (Table 1). At 160 days post-surgery, no significant differences between sham and TBI animals were evident. Notwithstanding these sex differences suggesting a greater impact on female well-being, our scoresheet detected suffering or pain during less than two weeks in both sexes. Welfare scores were then similar between sham and TBI animals, indicating that suffering, if present at these later time points, was not detectable by our scoring method. Results of score from each item can be found in Supplementary Table S1.

The scoresheet was also used to decide on animal care. For example, analgesic treatment with buprenorphine was administered during the first 24 h after injury, and was prolonged or reinstated if reaction to handling, grimace scale and/or mouth injury scored ≥ 10 . This happened during the first week for 12/15 animals with severe TBI (all female, 4 males), but only for one sham female. In addition, subcutaneous saline or glucose injections were decided if dehydration or body weight scored ≥ 10 , which happened in 12/15 TBI animals (all females, 4 males) and never for sham animals in the two weeks after surgery. Rats in which visual inspection revealed abnormally long incisors and displaying a welfare score ≥ 5 had their incisors filed. This happened in 13/15 animals (all females, 5 males) at 4 days, 9/15 animals (6 females, 3 males)

at 1 week, 7/15 animals (4 females, 3 males) at 28 days and 3/15 (1 female, 2 males) at 160 days after the TBI.

[Insert Figure 3]

[Insert Table 1]

Inter-rater reliability and an alternative scoresheet

Our scoresheet was validated across three evaluators with different levels of education and lab experience. Scoring took about 3 min per cage of two animals, and was very consistent among the three experimenters for 22/23 TBI animals, with only one female receiving high scores >90 by two experimenters, but not the third during the first four days post-surgery. Overall, even taking this animal into account, the intraclass correlation coefficient was 0.89 (0.88–0.91) (Figure 4(a) and supplementary figure S1).²⁴

We also applied an alternative scoresheet used for brain injury.¹³ Using this scale, 13/15 TBI animals (8 females, 5 males) that recovered a normal condition within two weeks reached a total score that would have triggered euthanasia from 1–4 days after surgery (Figure 4(b-c)) due to maximal score for spontaneous behavior (1 female), and weight loss (2 females, 2 males), and/or maximal overall score (12 animals, 7 females, 5 males). Among these animals, all but two recovered normal scores ≤ 10 , 28 days after TBI (except one male that scored 12, and one female at 30), and all were ≤ 5 after 160 days, indicating that suffering was no longer detected in these animals in the long-term. Results from each item can be found in Supplementary Table S2.

[Insert Figure 4]

Discussion

The presently introduced welfare scoresheet was developed specifically for assessing animal suffering after severe TBI. It includes general wellbeing items, such as weight, hydration, fur condition, and interactions with conspecifics, experimenters and toys, as well as TBI-associated items, such as respiratory function, gait, and the presence of wounds. The LFP method often induces transient respiratory dysfunction and mimics systemic complications in human patients. Gait deficits in TBI models parallel motor deficits²⁵⁻²⁷ and immobility/paralysis²⁶ in human patients. These deficits are not apparent in many rodent models of human disease, and should be specifically scored after TBI. In addition, TBI model rats should be monitored for the presence of wounds, excessive incisor growth, reduced food intake, weight loss, and a poor general condition. This scoresheet took only a few minutes to complete, and was implemented with a high degree of correlation across evaluators, even with different backgrounds in education and lab experience, suggesting high robustness.

Welfare scores took longer to normalize for females than males, including a longer lasting impact on grooming, dehydration, and interaction with environmental enrichment products. In humans, a meta-analysis suggested women may fare worse than men following mild–moderate TBI, but may fare better than men following severe TBI.²⁸ Females have been reported to fare better than males in some animal models.²⁸⁻³⁰ Prior examination of sex differences in outcomes of moderate-injury LFP model rats have also revealed higher mortality but smaller contusions in females.³¹⁻³³ Here, worse outcome in females was observed, but was also associated with a higher pressure applied to the dura. This result suggests that biomechanical factors, such as a smaller intracranial volume in females,³⁴ should be taken into account when assessing sex differences in TBI outcome, for example by comparing female and male rats of similar body weight (but different ages). Further studies are therefore required to better understand the sexual dimorphisms that may contribute to differences in TBI outcome.

To our knowledge, there are no specific studies applying a welfare scoresheet to a severe TBI model. An alternative scoresheet by Pinkernell et al. was designed for brain injury in general, but led to high scores and would have led to euthanasia in most of our animals, due to weight loss (>20%), immobility scores, and/or epileptic seizures.^{35, 36} Unspecific scoresheets and poorly adapted endpoints therefore have the potential of leading to unnecessary euthanasia of the animals.

Notably, we observed rapid recovery to normal welfare scores within two weeks of LFP. Severe TBI was demonstrated by loss of consciousness for >1 h, respiratory arrest lasting 10–30 s with cyanosis, and heart rate modifications lasting ~5 min. The transience of effects on welfare scores in our rats differs from the long-term cognitive problems and disability found in 60–65% of patients with severe TBI,^{2, 37} perhaps due to a higher degree of neuroplasticity and neurogenesis^{38, 39} as well as accelerated timescales of metabolic, inflammatory, and axonal dysfunctions in rodents.⁴⁰ Our study was limited to welfare assessment, and thus we did not track behavioral deficits or neurological lesions that might persist beyond 160 days.

Most importantly, data collected with this welfare scoresheet can provide information relevant to the ethical justification of animal use in TBI research. Careful rating of procedures in terms of animal welfare and injury severity is needed to clarify benefits to society versus hardship for animals. The present data demonstrate that LFP-induced severe TBI does not induce long-lasting distress or suffering that could be detected by our scoresheet. By principle, any unnecessary suffering should be avoided in animal research, and when suffering is inevitable, its impact should be carefully weighed against expected benefits to society. Careful characterization of animal welfare using a scoresheet precisely adapted to TBI is therefore a pre-requisite for accurate judgement of the costs of animal research on severe TBI by ethical committees, and supports a justified use of rodents in such research projects. Our scoresheet is also helpful for defining precise humane endpoints for animals with severe TBI in accordance

with the 3R criteria.⁷ We propose that a weight loss trigger for euthanasia be 30% rather than the common 20% limit^{13, 41} because 20~30% weight loss is common in moderate-to-severe TBI³⁵, and in our study, animals that lost up to 30% of their body weight recovered their normal weight within two weeks if other welfare indicators were improving. This higher limit would prevent unnecessary euthanasia and thus could reduce the total number of animals used in TBI projects. This study can provide experimental evidence for discussions with ethical committees worldwide, and possible adjustments of endpoints for animals with severe TBI. Other humane endpoints are respiratory dysfunction, total paralysis, irreversible infection, untreatable wounds, and a total score of ≥ 90 on our welfare scoresheet. Optimal adjustment of humane endpoints is key to the reduction of the number of animals included in experimental groups, as well as the refinement of procedures that may induce suffering.

Finally, welfare scores obtained during the first week following TBI could also provide an indication of the severity of the trauma, in addition to shockwave intensity, loss of consciousness indicated by RCS, withdrawal and righting reflexes recovery, and possible neurological lesions apparent on MRI or histological images. Mild, moderate and severe TBI are still poorly defined in animals, and it is possible that this scoresheet could participate in a better characterization of TBI severity, by taking into account animal welfare.

Study limitations

This study was performed using a limited sample size, and the scoresheet presented here has not been validated by multiple research centers. Blinding the scorers was not possible due to the large difference in welfare between sham and TBI animals during the first weeks following surgery. This could have potentially impacted the reliability of our scoring at later time points. We should also mention possible neurological effects on repeated anesthesia in animals

requiring frequent incisor filing. We are aware that some but not all ethical committees might allow an endpoint definition at 30% weight loss, depending on national regulations and local ethical sensitivities.

Conclusion

This study proposes a reproducible and easy-to-use welfare scale adapted to severe TBI in rodents. In addition to providing semi-quantitative estimates of animal welfare to refine TBI models, this scoresheet demonstrated that suffering is apparent during a transitory period following TBI, but no longer detected in the long term, with animals recovering normal welfare within less than two weeks.

Author contributions

CA and SM designed the welfare scoresheet. CA performed in vivo experiments. CA, LA and FC scored animals and retrieved data. CA, SM and BB contributed to the design of statistical analyses. CA and SM wrote the manuscript, with contributions made by all of the other authors.

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Disclosure statement

No competing financial interests exist.

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Figure legends

Figure 1. Scoresheet for severe traumatic brain injury. 0–10 normal physical condition, no pain; 11–60 presence of discomfort indicating a need for sustained observation and increased monitoring, moistened, palatable food, analgesia, and subcutaneous saline or glucose; 61–89 significant suffering and pain indicating a need for compensatory measures, subcutaneous glucose, veterinary advice, and consideration of euthanasia; ≥ 90 severe suffering indicative of a humane endpoint. T0, pre-surgery weight; Tx, daily weight.

Figure 2. Return to consciousness after severe traumatic brain injury (TBI). Hindpaw withdrawal reflex (a) and righting reflex recovery (b). (c) Evolution the Rat Coma Scale (RCS) scores. The dotted line is the mean score for sham rats, and solid line is the mean score for TBI rats. The black dotted line is the maximum score of sham rats. (d) Time to return to full consciousness assessed by the RCS. Values are shown as median [inter-quartile range]. Sham females (F) $n = 10$, TBI F $n = 8$, sham males (M) $n = 9$, TBI M $n = 7$; NS, non-significant (Student's t-test); # represents a significant interaction between sex and recovery time, $p < 0.05$, female vs. male comparison; *** $p < 0.001$, TBI vs. sham comparison (two-way ANOVA followed by Mann-Whitney U post-hoc test).

Figure 3. Animal welfare after severe TBI. (a) Evolution of welfare scores in sham and TBI animals. The dotted line is the mean score for sham rats, and solid line is the mean score for TBI rats. The black dotted line is a humane-endpoint threshold. (b) Area under curve of welfare scores during the first 12 days. (c) Percent changes in body weight during 160 days after TBI. The black dotted line is the basal value. Values are shown as median [inter-quartile range].

Sham females (F) $n = 10$, TBI F $n = 8$, sham males (M) $n = 9$; TBI M $n = 7$; NS F vs. M; *** $p < 0.001$ TBI vs. Sham comparison (two-way ANOVA followed by Mann-Whitney U post-hoc test).

Figure 4. Inter-rater reliability and excess mortality with alternative welfare scoresheet. (a) Comparison between three independent experimenters. Each point corresponds an animal's daily score (x: experimenter 1, y: experimenter 2, z: experimenter 3). (b) Maximal scores and (c) temporal evolution obtained with an alternative welfare scoresheet.¹³ The dotted line is the mean score for sham rats, and solid line is the mean score for TBI rats. The black dotted line represents the humane endpoint triggering euthanasia in the alternative scoresheet. Values are shown as median [inter-quartile range]. Sham females (F) $n = 10$, TBI F $n = 8$, sham males (M) $n = 9$, TBI M $n = 7$.

Table 1. Return to basal levels of scoresheet criteria and minimum weight after the severe traumatic brain injury (TBI). Values are presented as median [inter-quartile range]. Repeated Mann-Whitney U tests ($p < 0.025$).

Table 1

Parameter	Females (F)		Males (M)		<i>p</i> value			
	Sham (n = 10)	TBI (n = 8)	Sham (n = 9)	TBI (n = 7)	Sham (F vs. M)	TBI (F vs. M)	Female (Sham vs. TBI)	Male (Sham vs. TBI)
Welfare (return to 10, days)	2.0 [0.0–3.0]	16.5 [10.5–24.5]	2.0 [0.0–3.0]	6.0 [4.0–12.0]	0.997	0.0496	< 0.0001	0.0007
Silky coat (days)	1.5 [1.0–3.0]	10.5 [7.0–26.8]	1.0 [0.5–2.0]	4.0 [3.0–8.0]	0.369	0.0185	< 0.0001	0.0044
Grimace scale (days)	0.0 [0.0–1.0]	9.0 [6.0–19.0]	1.0 [0.0–1.0]	4.0 [2.0–10.0]	0.448	0.179	0.0001	0.0083
Respiration (days)	0.0 [0.0–1.0]	2.5 [2.0–3.0]	0.0 [0.0–1.0]	2.0 [1.0–5.0]	> 0.999	0.6115	0.0002	0.0122
Spontaneous behavior (days)	0.0 [0.0–0.3]	1.0 [0.0–2.0]	0.0 [0.0–0.0]	0.0 [0.0–0.0]	0.4737	0.0741	0.0656	0.4375
Response to handling (days)	0.0 [0.0–1.3]	3.5 [3.0–4.8]	0.0 [0.0–1.0]	2.0 [1.0–11.0]	0.6213	0.4359	0.0002	0.0023
Locomotion (days)	0.0 [0.0–0.0]	7.5 [6.0–13.8]	0.0 [0.0–0.5]	3.0 [3.0–8.0]	0.0582	0.0559	< 0.0001	0.0002
Interaction with environment (days)	0.0 [0.0–0.0]	1.0 [0.3–2.8]	0.0 [0.0–0.0]	0.0 [0.0–0.0]	0.4737	0.0250	0.0015	> 0.999
Wound healing (days)	0.0 [0.0–0.0]	1.5 [0.3–3.0]	0.0 [0.0–0.0]	1.0 [0.0–3.5]	0.4737	0.6767	0.0015	0.0799
Hydration (days)	0.5 [0.0–1.3]	15.5 [9.0–23.3]	1.0 [0.5–1.0]	7.0 [4.0–8.0]	0.4287	0.0065	< 0.0001	0.0038
Body condition (days)	2.5 [0.0–3.3]	12.5 [9.0–23.5]	1.0 [0.0–3.5]	6.0 [3.0–9.0]	0.9689	0.0109	< 0.0001	0.038
Weight (days)	0.0 [0.0–0.8]	16.0 [7.5–24.5]	0.0 [0.0–3.0]	8.0 [3.0–11.0]	0.3638	0.0774	< 0.0001	0.0155
Maximal weight loss (%)	3.2 [1.3–5.2]	16.3 [11.1–22.8]	3.3 [1.9–7.2]	14.2 [6.2–22.6]	0.7802	0.5358	0.0003	0.0052

Animal ID: _____ Date/hour of surgery: _____
 Protocol: _____ Group: _____ Pre-surgery body weight (g): _____

		Date / hour
REMOTE OBSERVATION		
General condition, appearance	Well groomed	0
	Lack of grooming, slight presence of porphyrin on the eyes	2
	Piloerection, unkempt fur, marked porphyrin stains	10
Grimace scale	Normal	0
	Moderate signs	10
	Severe signs	15
Breathing rate	Normal	0
	Dyspnea, hypoventilation	10
	Difficult and marked, cyanosis	90
Spontaneous behavior	Normal social interaction	0
	Reduced social interaction	2
	Refuses social interaction, completely isolated	5
EXAMINATION DURING HANDLING		
Reaction to handling	Normal curiosity, approaches experimenter	0
	Slightly reluctance to contact	5
	Prostration, apathy, avoids contact	10
	Total paralysis (>2 days), pre-comatose, lethargy	90
Locomotion / gait	Normal gait	0
	Slight loss of balance, skims over the ground	2
	Strong loss of balance, atonic	10
Interaction with environment, enrichment	Normal use of enrichment, nesting	0
	No use of enrichment, no nesting behavior	5
PHYSIOLOGICAL PARAMETERS		
Mouth injury, post-surgical sequelae	No sign of infection, clean wound	0
	Benign malocclusion, scratches around the scar	5
	Severe malocclusion, injury to mouth and lips, absence of wound healing	10
	Irreversible infection spread, purulence.	90
Hydration (skin pinch)	Normal <1 s	0
	≤2 s	5
	>2 s	10
Body condition scoring	Good	0
	Fair	5
	Emaciated	10
Body weight loss % $\frac{T_0 - T_x}{T_0} \times 100$	Body weight (g)	
	0–5	0
	6–10	2
	11–15	5
	16–20	10
	20–25	15
	26–29	20
≥30	90	
		Total:

Notes (evolution injury, seizure, immobility...):

Figure 2

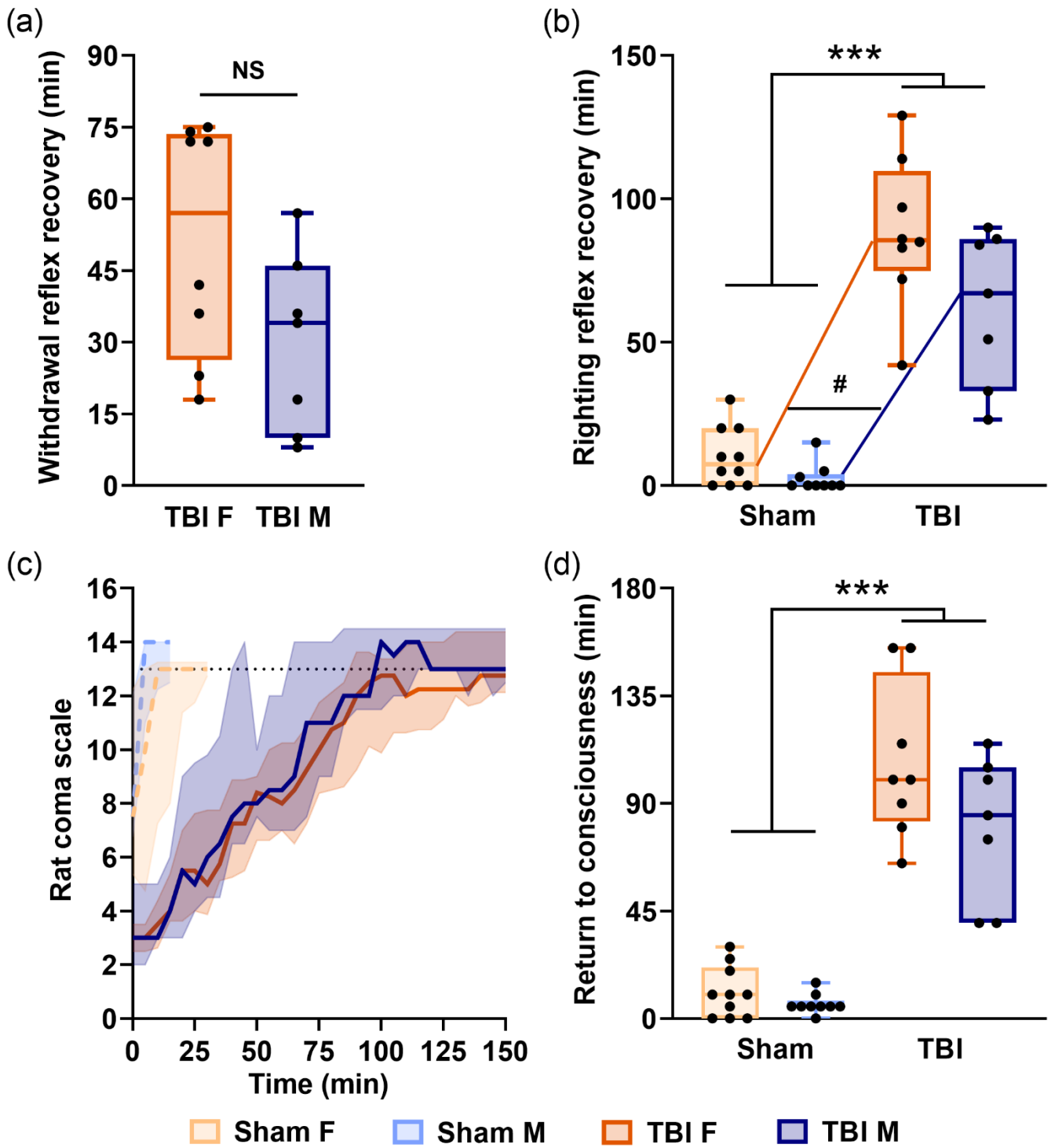


Figure 3

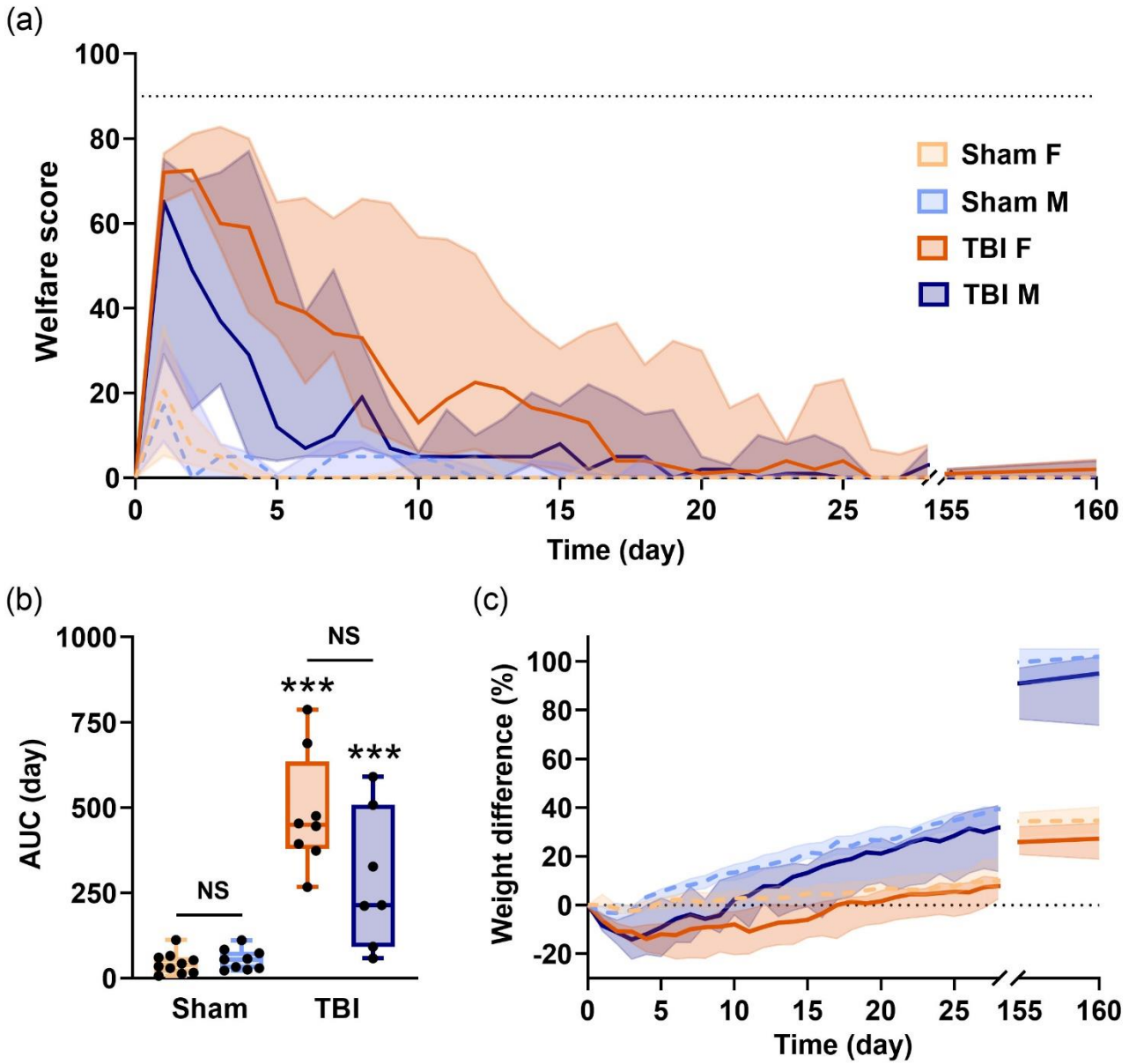


Figure 4

