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**Studies on long term behavioural changes in group-housed rat models of brain and spinal cord injury using an automated home cage recording system**

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1 **Abstract:**

2 Background: Neurotrauma patients face major neurological sequelae. The failure in the  
3 preclinical-to-clinical translation of candidate therapies could be due to poor evaluation of  
4 rodent behaviours after neurotrauma.

5 New Method: A home cage automated system was used to study the long term behaviour  
6 of individual rats with traumatic brain injury (TBI), spinal cord injury (SCI) and non-CNS  
7 injured controls, whilst group-housed in their home cages. Naïve rats were used as  
8 baseline controls. Automated locomotor activity and body temperature recordings were  
9 carried out 24 h /day for 3 days/week during 12 weeks post-injury. Behavioural patterns,  
10 including aggression, rearing, grooming, feeding and drinking were analysed from  
11 automated video recordings during week 1, 6 and 12.

12 Results: SCI animals showed a lower locomotor activity compared to TBI or control  
13 animals during light and dark phases. TBI animals showed a higher aggression during  
14 the dark phase in the first week post-injury compared to SCI or control animals. Individual  
15 grooming and rearing were reduced in SCI animals compared to TBI and control animals  
16 in the first week post-injury during the dark phase. No differences in drinking or feeding  
17 were detected between groups. Locomotor activity did not differ between naïve male and  
18 female rats, but body temperature differ between light and dark phases for both.

19 Standard methods: Injury severity was compared to standard SCI and TBI behaviour  
20 scores (BBB and mNSS, respectively) and histological analysis.

21 Conclusions: This study demonstrates the practical benefits of using a non-intrusive  
22 automated home cage recording system to observe long term individual behaviour of  
23 group-housed SCI and TBI rats.

24

## 25 **Introduction**

26 Traumatic injuries are the single greatest cause of lost human potential worldwide, and  
27 traumatic brain injury (TBI) and spinal cord injury (SCI) are associated with death or  
28 lifelong disability (1). Furthermore, their incidence is increasing, due to the global aging  
29 of the population.

30 TBI (2) and SCI (3) involve two distinct phases of injury – the primary injury caused  
31 immediately by the mechanical insult, and the secondary injury, evolving over time  
32 through a cascade of vascular, cellular and biochemical events (4). Despite advances in  
33 pre-hospital trauma management, there are no effective treatments to reverse the primary  
34 CNS damage and most therapeutic developments focus on modulating the progressive  
35 secondary injury, to support regeneration of the injured CNS.

36 Despite a large number of preclinical studies, generally with apparent robust validity,  
37 treatments have shown very limited impact in the clinic. Yet, research on CNS injury must  
38 advance and *in vivo* modelling still remains an instrumental tool for mechanistic studies  
39 on injury pathophysiology (5).

40 Assessment of functional impairment remains critical for CNS modelling in which motor  
41 and/or sensory recovery tests are often used. The BBB locomotor scale is a standard  
42 kinematic measure used to assess hindlimb motor recovery following thoracic SCI in rats

43 (6). Other tests such as the grip strength test, which measures muscle strength (7), or the  
44 Hargreaves hot plate or von Frey filaments tests, can also be used in SCI models to  
45 assess thermal hyperalgesia and mechanical allodynia, respectively (7, 8). The modified  
46 neurological severity score (mNSS) is commonly used in rodent models of TBI to evaluate  
47 motor, sensory, proprioceptive and reflex behaviours (9, 10).

48 However, these behavioural tests are biased towards assessing task driven and not  
49 spontaneous behaviour, which may poorly reflect translatable outcomes with therapeutic  
50 impact (11). Most studies implement test batteries which have many confounders, such  
51 as test time and order, environment enrichment and acclimatization time (12).  
52 Furthermore, most behavioural tests involve momentarily removing the animal from its  
53 home-cage and social group and exposure to a new and unfamiliar environment (13, 14)  
54 which is then confounded further but the impact of different handlers and handling  
55 expertise. Also, many of these tests only allow for a “snap-shot” assessment of daily  
56 behaviour, missing infrequent disease phenotypes that happen outside a window of  
57 observation (e.g. seizures at night) (15). Moreover, rodents are crepuscular (16, 17), so  
58 solely assessing them during the working day of a research scientist will very likely mask  
59 the full extent of relevant neurobehavioural changes. Thus, classical assessment of  
60 rodent behaviour needs to be complemented with other unforced and non-stimulated  
61 automated assessment approaches in the home cage over long time intervals. This is  
62 particularly relevant to investigate the impact of injury on cognitive and social functions  
63 and the potential therapeutic benefits in neurotrauma models.

64 Body temperature, which is infrequently studied can be a valuable indicator of  
65 homeostasis during surgery and post-operative care, could directly impact recovery from

66 CNS injury. Furthermore, a large variability in body temperature, due to the inflammatory,  
67 cardiovascular and/or shock response, could impact drug testing outcomes (18).  
68 Therefore, regular monitoring across the light and dark phases is critical in studies  
69 involving neurotrauma models.

70 Recently, one technology available to researchers is the home cage analysis (HCA),  
71 which facilitates the assessment of caged animals in their undisturbed 'home'  
72 environment. HCA systems utilise a variety of technological modalities, including video  
73 technology, infrared (IR) sensors and telemetry (17). Most systems rely on one-or-two of  
74 these approaches, and have been successfully used to characterise individual  
75 behavioural profiles in rodent models of Huntington's disease and prion diseases (17),  
76 and also some studies have been reported in single housed neurotrauma mouse models(  
77 [REF-Ping](#)). Few systems support long-term monitoring and data analysis on grouped  
78 housed animals.

79 Recently, an automated home cage recording system was developed by Actual Analytics  
80 Limited in collaboration with the National Centre for the 3Rs (NC3Rs), which was capable  
81 of capturing individual temperature and behavioural data of rodents group-housed in  
82 normal home cages over long periods of time (12, 19).

83 To investigate the utility of this automated home cage recording system in traumatic CNS  
84 injury, we used this recording system to monitor changes in the behavioural phenotype  
85 of group-housed rat models of TBI and SCI, during sub-acute and chronic post-injury  
86 phases. Automated body temperature and basic behavioural monitoring was completed  
87 using non-invasive, automated telemetry and digital data collection throughout both light  
88 and dark phases for up to 12 weeks post-injury. Subsequent manual review of

**Commented [JL1]:** PINg will you be ok to add the references:

<https://www.ncbi.nlm.nih.gov/pubmed/30176241>

Vu et al (2018) Transient disruption of mouse home cage activities and assessment of orexin immunoreactivity following concussive- or blast-induced brain injury

This study uses the Any-Maze cage (AMc) housing and activity monitoring, which is for a single mouse

<https://www.ncbi.nlm.nih.gov/pubmed/27073377>

\* Qu (2016) Automated monitoring of early neurobehavioral changes in mice following traumatic brain injury  
SmartCage system is a non-invasive home cage rodent behaviour monitoring system, which is for a single mouse

89 corresponding IR video data was completed to derive more complex neurobehavioural  
90 insights.

91

## 92 **Methods**

### 93 **Ethical statement**

94 All animal procedures were carried out under two Project Licences (PPL 70/8712 and  
95 PPL 70/7436) approved by the Animal Welfare and Ethical Review Body at Queen Mary  
96 University of London and the UK Home Office, in accordance with the EU Directive  
97 2010/63/EU. All animal facilities and suppliers have been approved by the UK Home  
98 Office Licensing Authority and meet all current regulations and standards for the UK. A  
99 total of 24 rats were used for the work described in the study, 18 of which underwent  
100 surgical recovery procedures. For this exploratory study we used n=6 animals per group,  
101 based on our previous efficacy studies using these neurotrauma models (20, 21) to  
102 provide a valuable discriminatory power of 80% with a significant level  $\alpha = 0.05$  to detect  
103 approx. 20% relative differences in behaviour and histological assessments as primary  
104 outcomes for our neurotrauma studies. Experimental planning for data randomization and  
105 blinding data acquisition and analysis was carried out following the ARRIVE guidelines  
106 (22).

### 107 **Animal housing and husbandry**

108 A total of 24 adult Sprague-Dawley rats (weight range 200 - 300 g; 9 - 10 weeks old at  
109 the start of the study) were obtained from Charles River Laboratories, Margate, UK.  
110 Health screens provided by the official vendor indicated that rats were free of known

111 pathogens in accordance with FELASA Recommendations for health monitoring of rodent  
112 colonies (23). Animals were housed in groups of 3 per Individually Ventilated Cage (IVC;  
113 Allentown Europe, UK), in a 12 h light dark cycle (06:30 - 18:30 light; 18:30 - 06:30 dark),  
114 with controlled room temperature ( $21 \pm 1$  °C) and relative humidity (40-60 %). The cages  
115 contained 1-1.5 cm layer of animal bedding (Lignocel®, Rettenmaier UK Ltd). Rats had  
116 access to food (Labdiet® EURodent 14% Diet 5LF2, LabDiet, Brentwood, Missouri, U.S.)  
117 and water *ad libitum*. Rats were allocated to cages on arrival and remained in the same  
118 social group throughout the study, including a 7 day acclimatization phase to the  
119 laboratory.

#### 120 ***SCI and TBI surgical procedures and in vivo experimental design***

121 Surgery was carried out in accordance with protocols reported previously (21, 24). All  
122 animals were anaesthetised intraperitoneally with ketamine (Ketaset®) (50mg/kg) and  
123 medetomidine (Domitor™) (0.2mg/kg), followed by subcutaneous administration of  
124 buprenorphine (Buprenex®) (0.1mg/kg) for prophylactic analgesia. For TBI surgery, the  
125 rat head was clipped, surgically scrubbed and subsequently secured to a stereotactic  
126 frame using mouth, nose and ear bars, before a sagittal incision was made through the  
127 scalp to expose the cranium. Utilising the PCI3000 Precision Cortical Impactor™  
128 (Hatteras Instruments, Cary, NC), a “closed” TBI was induced by directly delivering a blunt  
129 impact using a 5 mm diameter impactor tip to the right parietal bone, with the central  
130 coordinates set at -3.5 mm from bregma and -3.5 mm from the midline. The impaction  
131 was carried out using a 3.0 m/s velocity, a 3.0 mm impact depth, a 100 ms dwell time, at  
132 a 20° angle to the bone. Following impact to the skull, the scalp was sutured, and animals  
133 were placed in a warm incubator (27–28 °C) to recover. Reversal of anaesthesia involved



134 subcutaneous administration of atipamezole (Antisedan®) (0.1mg/kg). For SCI surgery,  
135 the anaesthetised rats underwent a midline incision through thoracolumbar fascia on a  
136 clipped and surgically scrubbed skin area, and the underlying muscles were pulled away  
137 from the T9– T11 spinous processes and laminae. The lateral aspects of the T9 and T11  
138 vertebral bodies and spinous processes were clamped to stabilize any movement of the  
139 spinal cord. A bilateral laminectomy was performed at T10, leaving the dura exposed but  
140 intact. After securing the spinal column, the PCI3000 Precision Cortical Impactor™  
141 (Hatteras Instruments, Cary, NC) was used with the following settings: a 2 mm impactor  
142 tip, 1.5 m/s velocity, 1.8 mm impact depth, and 100ms dwell time, at a 90° angle to the  
143 cord (24). Sham laminectomy animals underwent the same procedure as SCI-treated  
144 animals, excluding the contusion injury on the spinal cord. Upon completion of spinal  
145 surgery, the spinal fascia and muscle followed by the skin were sutured. Atipamezole was  
146 administered, and the rat was placed in a warm incubator to recover (27 – 28 °C). Finally,  
147 a radio frequency identity detection (RFID) chip was ‘injected’ subcutaneously into the  
148 right flank of each rat, to permit tracking by the ActualHCA system. During the  
149 postoperative recovery phase, all animals received buprenorphine (Buprenex®)  
150 (0.1mg/kg) analgesia together with saline, subcutaneously administered twice daily for 3  
151 days after surgery. Bladders were manually expressed twice a day for the SCI animals  
152 until return of bladder function (<2 ml of urine in early morning expression for three  
153 consecutive days).

154 The study was carried out in two consecutive periods of 12 weeks, for all experimental  
155 groups (SCI, TBI and non-CNS injured control; randomly n=3 per group) to reach a total  
156 of n=6 animals per group. Sex allocation was informed by the literature; female rats are

157 commonly used for SCI studies and male rats for TBI studies. To test for gender effect on  
158 locomotor activity and body temperature, 6 surgery-naïve control animals (n=3 males and  
159 n=3 females) were also used.

#### 160 **Conventional behaviour tests**

161 Using the Basso, Beattie, Bresnahan (BBB) locomotor rating scale, open field locomotion  
162 assessment was carried out daily during the first week post-injury and then once weekly  
163 over 11 weeks, to characterize the functional outcome after spinal injury in the SCI and  
164 non-CNS injured control group (Suppl. Fig. 1A). A modified neurological severity score  
165 (mNSS) was used to evaluate motor ability, balance and alertness during the first week  
166 post-injury in the TBI group (Suppl. Fig. 1B).

#### 167 **Histology**

168 At the end of the study (12 weeks post-injury) animals were deeply anaesthetized with  
169 sodium pentobarbital (50 mg/kg, i.p.; Sagatal, Rhone Merieux, Harlow, UK), and received  
170 a transcardiac perfusion with phosphate-buffered saline (PBS; 0.01 M, pH 7.4), followed  
171 by 4 % paraformaldehyde (PFA) in phosphate buffer (0.1 M, pH 7.4). Tissues were  
172 dissected out, post fixed in 4 % PFA for 2 h, and cryoprotected in 20 % sucrose in 0.1 M  
173 phosphate buffer at 4°C until further processing. Serial 20 µm coronal sections of whole  
174 brain and horizontal sections of spinal cord (extending approximately 1 cm rostral and 1  
175 cm caudal from the contusion centre) were cut using a cryostat for histology.  
176 Representative serial sections were processed for Cresyl Violet (Nissl) staining. All brain  
177 tissue staining was performed between bregma - 1.28 mm and bregma -2.34 mm, where  
178 the lesion was located. Spinal cord staining was performed between the dorsal contusion  
179 site and approximately half the cord thickness.

180 **Automated home cage recording system**

181 The automated home cage recording system (Home Cage Analyser (ActualHCA™)  
182 system, Actual Analytics Ltd, UK) was used and specially fitted for standard IVCs  
183 (Allentown). Each automated home cage recording system was placed on a bespoke  
184 frame to support the placement of the IVC directly on top of the baseplate RFID reader.  
185 The infrared HD video camera and mini-computer for data recording were placed on a  
186 side slot frame facing one of the long sides of the IVC. On the top of the frame, an infrared  
187 lighting panel was placed above the top of the IVC (see Figure 1 for representation of the  
188 HCA system set up).

189 RFID transponders for animal identification and temperature measurements were  
190 supplied by BioMark (Boise, ID83702, US). The Biomark BioTherm13 Passive Integrated  
191 Transponder (PIT) is an RFID device with a  $2.1 \pm 0.1$  mm diameter and  $12.0 \pm 0.4$  mm  
192 length applied for subcutaneous implantation (ISO standards 11784/11785). All devices  
193 were factory calibrated (temperature range 33.0 - 43.0 °C). The baseplate RFID reader  
194 was designed to work with BioTherm13 RFID transponders.

195 The baseplate RFID reader consists of an array twelve transceiver coils, situated in  
196 waterproof casing underneath the cage. Each individual coil covers a separate 12 x 12cm  
197 square region underneath the cage floor and can detect the presence of an RFID chip up  
198 to a height of 13 cm. The twelve coils are arranged in a regular 3 x 4 grid spanning a total  
199 area of 36 x 48 cm, allowing motion in the plane of the cage floor (30 cm x 41 cm) to be  
200 recorded. Activity in the vertical plane (e.g. rearing) is not captured by the baseplate  
201 reader, but can be extracted from the concurrent video recorded (33). Rats are detected  
202 by the nearest antenna reading the ID and temperature from the RFID transponder.

203 Intermediate positions between adjacent antennae are sorted by applying a filtering  
204 correction algorithm (33). When more than one animal is detected by the same antenna,  
205 the greater strength signal corresponds to the closest animal (19). Continuous video  
206 recordings were acquired by using infrared (IR) LEDs at 860 nm wavelength to illuminate  
207 the cage from above, and USB 3.0 cameras with matched 4.5 mm lenses and daylight  
208 filters (700 nm cut-off) were used to capture grayscale videos at 25 fps at HD (720p)  
209 resolution.

#### 210 **Data acquisition**

211 At a pre-defined time, animals were transferred into the automated home cage recording  
212 system (Fig. 1). Three animals per experimental group (SCI, TBI, control) were studied  
213 weekly for a 12 week study period, since the automated recording system functions  
214 optimally when only tracking 3 animals within the same cage (25). For the CNS-injured  
215 and non-CNS-injured control animals, RFID data (animal ID, locomotor activity and  
216 temperature) and IR video data were captured 24 h/day, 3 days/week, for up to 12 weeks.  
217 Naïve non-surgery animals (n=3 male in 1 cage; n=3 female in 1 cage) were studied for  
218 5 days only.

219 Actual HCA Capture™ software (Actual Analytics Ltd, Edinburgh, UK) was used to  
220 manage data capture and system calibrations, before IR video and matched baseplate  
221 RFID data were stored to a local hard drive. Throughout each experiment, data analysis  
222 was carried out using a time-binning of 5 min and video segment length of 30 min. Time-  
223 binning indicates the duration of time represented by each datum in the data analysis  
224 report (e.g. 100 mm travelled in 5 min).

#### 225 **Data sampling and analysis**

226 RFID data were pooled and analysed using the Actual HCA Analyser™ software (Actual  
227 Analytics Ltd, Edinburgh UK). We plotted ‘transitions’ against time as a measure of  
228 ‘Locomotor Activity’ (33). Specifically, one transition defines the movement of an animal’s  
229 RFID chip across the electromagnetic field boundary between two adjacent antennae.  
230 This measure directly correlates with locomotion activity and distance. Subcutaneous  
231 body temperature was also recorded via RFID chips.

232 Automatic RFID recordings aligned with the IR video recordings (~13.72 GB data per day  
233 for a single cage of 3 animals in VLC media and HDF5 file formats) were used to visually  
234 investigate selected behaviours (aggression, grooming, rearing, feeding and drinking).  
235 With over 216 days (~23 days/week x 12 weeks x 6 groups = 216 days) of IR video  
236 footage recorded, data sampling was required. RFID activity automatic data was tracked  
237 per group per week 1, 6 and 12, to represent early subacute, late subacute and chronic  
238 phases of injury, respectively (26, 27). Periods showing larger activity patterns were  
239 selected for visualization of the video recorded data to better identify the display of  
240 behavioural expressions (Suppl. Fig. S2). The five behaviours of interest were selected  
241 based on their frequency of occurrence, after reviewing preliminary IR video footage and  
242 ease of detection and insight into regular behaviour: aggression, grooming, rearing,  
243 feeding and drinking. Further characterization and details are summarized in Fig. 2.

244

#### 245 **Statistical analyses**

246 All the behaviour (the primary study endpoint), was assessed blind, with the researcher  
247 unaware of the allocated intervention. Data from the two 12 week recording periods were

248 pooled together such that n=6 per experimental group, with n=3 animals grouped per  
249 cage was analyzed.

250 Locomotor activity data were not normal distributed, so were analyzed using Kruskal-  
251 Wallis Test (with Pairwise Mann-Whitney U test post hoc analysis tests). Temperature  
252 data were normally distributed, so were analyzed using two-way ANOVA (with Tukey's  
253 post hoc analysis tests). Data were shown as mean and standard error of the mean (SEM)  
254 and comparisons were selected as statistically significant at  $p < 0.05$ . These analysis  
255 were performed in R v3.5.1.

256 For the specific behavioural expressions (i.e. aggression, individual grooming, rearing,  
257 feeding and drinking data acquired in combination from the RFID digital data with the IR  
258 video recordings) mean  $\pm$  SEM were calculated for the duration of time and each  
259 behaviour was expressed during a sample 5 min period per 12 h light or dark phase per  
260 group per week (pgpw). Temporal changes in behavioural phenotype within each group,  
261 and differences in phenotype between groups at defined time-points, were each assessed  
262 by two-way ANOVA and Tukey's post-hoc test when statistical significance was identified.  
263 Statistical significance was set at  $p < 0.05$ . These analyses were performed using Prism,  
264 version 7.03 (GraphPad Software Inc., San Diego, CA).

265 A correlation analysis (Ping\_which test?) was used to assess the association between the  
266 information provided by the automated RFID recordings (Nm of transitions indicating  
267 locomotor activity; including light and dark phase activity analysis) and the conventional  
268 behaviour tests, and also the histological endpoints (spinal cord cavity and ventricle sizes  
269 for the SCI and TBI groups, respectively). These analyses were performed using Prism,  
270 version 7.03 (GraphPad Software Inc., San Diego, CA).

## 271 **Results**

### 272 **Subacute behavioural analysis of naïve animals**

#### 273 *Locomotor activity and temperature data demonstrating circadian pattern*

274 Using the automated RFID digital data from the automated home cage recording system,  
275 locomotor activity and temperature data for naïve male and female rats were collected  
276 from 5 days (24/7 recordings; 3 rats/ group). Naïve animals showed no significant  
277 difference in locomotor activity and body temperature between male and female in the  
278 light or dark phases (Fig. 3A-D). Qualitative observations showed spikes of increased  
279 locomotor activity during the dark phase for both male and female groups, but no  
280 significance was observed. (Fig 3A-B). A circadian light/dark pattern was statistically  
281 significant for both the body temperature of male and female rats (male:  $p = 0.001$ , female:  
282  $p < 0.001$ ) (Fig. 3D).

### 283 **Locomotor activity changes in SCI, TBI and Control animals**

#### 284 *SCI induces a reduction on locomotor activity during the first week post-injury*

285 The automated RFID digital activity data from the automated home cage recording  
286 system (data plotted 24/7 for the 3 days post-injury; 6 animals/group), showed a  
287 significant reduction on the locomotor activity of SCI animals during the first week post-  
288 injury relative to TBI ( $p = 0.045$ ) and control animals ( $p = 0.045$ ) (Fig. 4 & 5A).  
289 Interestingly, at week 6 post injury, there was a significant increase on the locomotor  
290 activity of SCI animals compared to TBI ( $p = 0.026$ ) and control animals ( $p = 0.039$ ) (Fig.  
291 4 & 5B). However, at week 12 post injury, no significance between the groups were  
292 observed (Fig. 5C). When the light and dark phases were analysed, all CNS injury groups

293 showed a significant difference in locomotor activity at weeks 1, 6 and 12 post injury (Fig.  
294 5A-C). Temporal analysis of the locomotor activity exhibited a significant decrease in SCI  
295 animals at week 1 and 12 compared to week 6 post injury in the light phase ( $p = 0.013$   
296 and  $p = 0.039$ , respectively)(Fig. 5D). Furthermore, locomotor activity for SCI in the dark  
297 phase at week 1 was significantly decreased compared to both weeks 6 and 12 post injury  
298 ( $p = 0.003$ ) (Fig. 5D). Interestingly, locomotor activity was not altered in TBI group (Fig.  
299 5E), but the non-CNS injured control group exhibited a significant decrease in locomotor  
300 activity at week 12 compared to week 1 in the light phase ( $p = 0.007$ ), and a significant  
301 increase in locomotor activity at week 6 compared weeks 1 and 12 in the dark phase ( $p$   
302  $= 0.007$ ) (Fig. 5F).

303

304 *SCI and TBI induces a reduction in body temperature at light phase of week 6 and 12*  
305 *post-injury*

306 Subcutaneous body temperature recordings from automated RFID data (data plotted 24/7  
307 for the 3 days post-injury; 6 animals/group) showed no significant changes between the  
308 CNS injury groups during week 1 and 12 post injury (Fig 6 & 7A, C). Interestingly, at week  
309 6 post injury, body temperature was significantly altered between the light and dark phase  
310 for SCI ( $p = 0.029$ ) and TBI ( $p = 0.018$ ), but not the control group (Fig. 7B). Temporal  
311 analysis of body temperature in SCI and TBI group exhibited a significant reduction in  
312 body temperature at weeks 6 and 12 at light phase and between light and dark phases  
313 (Fig. 7D & E). In the non-CNS injured control group, no significant alteration in body  
314 temperature were observed between light and dark phase and in any temporal manner  
315 (Fig. 7F).



316

317 **Temporal changes in selected behavioural phenotype in SCI, TBI and Control**  
318 **animals**

319 *Feeding and drinking behaviour did not change over time with CNS injury*

320 Using the combination of RFID and IR Video data at week 1, 6 and 12 post-injury, detail  
321 analysis of recordings at the duration of time in which rats spent feeding and drinking  
322 were carried out. This was a proxy measure of food consumption and water intake,  
323 respectively. No significant changes in the expression of either feeding or drinking were  
324 observed between weeks 1, 6 and 12 for SCI, TBI and non-CNS injured control animals  
325 (Fig. 8A-F). These data suggest that the CNS injuries in these animals do not significantly  
326 limit the animals' ability to feed and drink *ad libitum*.

327

328 *Grooming behaviour was lowest in SCI rats in the first week after CNS injury*

329 The duration of time rats spent in individual grooming, by manual curation of the RFID  
330 and IR Video data at weeks 1, 6 and 12 post-injury, as proxy measure of self-maintenance  
331 were manually analysed. At week 1 post-injury, mean dark phase grooming was  
332 significantly lower in the SCI than the non-CNS injured control group ( $p = 0.044$ ) (Fig.  
333 8G). Additionally, a trend difference ( $p = 0.063$ ) in dark phase grooming behaviour was  
334 also shown in TBI vs. SCI animals (Fig. 8G). However, thereafter at weeks 6 and 12, no  
335 significance difference in grooming for any groups were observed (Fig. 8H-I). These data  
336 suggest SCI interferes with the animals' grooming activity during the first week post injury.

337

338 *Rearing behaviour activity increases over time after SCI*

339 Using the RFID and IR video data, we manually analysed the duration of time rats spent  
340 rearing as a proxy measure of hind limb motor function and possibly higher interest (e.g.  
341 exploration, information gathering). Not surprisingly, at week 1 post injury, SCI animal  
342 with hindlimb paralysis had significantly fewer rearing than the control animals at the dark  
343 phase ( $p = 0.037$ ) (Fig. 8J). No significance was observed in mean duration of rearing at  
344 weeks 6 and 12, between the SCI, TBI and non-CNS injured control animals (Fig. 8K-L).  
345 Temporal rearing activity from week 1 to week 12 did not exhibit any significant difference  
346 within the non-CNS injured control or the TBI group (Fig. 9A & C). However, during the  
347 dark phase, SCI animals exhibited a significant increase rearing in week 12 when  
348 compared to week 1 ( $p = 0.012$ ) (Fig. 9B). These data suggest SCI limits the animals from  
349 carrying out rearing activity during the first week post injury.

350

351 *Aggression was significantly higher in TBI animals early after injury*

352 Using the RFID and IR Video data we manually recorded the duration of time that rats  
353 demonstrated aggression, as a proxy measure of antagonism. In week 1 post injury, the  
354 mean duration of aggressive behaviour was significantly higher in the TBI than SCI or  
355 non-CNS injured control groups, during the dark phase ( $p < 0.001$ ,  $p = 0.004$ ,  
356 respectively)(Fig. 9D). Also, aggression in TBI was higher in the dark phase than light  
357 phase at 1 week post injury ( $p < 0.001$ ) (Fig. 9D). At week 6 post injury, dark phase  
358 aggression in the TBI group was also significantly higher than SCI group, and higher than  
359 in the light phase ( $p = 0.014$ ,  $p = <0.001$ , respectively) (Fig. 9E). At week 12 post injury,  
360 there was no significant difference in the expression of aggression between groups (Fig.

361 9F). Temporal aggression activity in the TBI group was significantly higher at dark phase  
362 week 1 post injury compared to light phase week 1 and dark phase week 6 and 12 ( $p$   
363  $<0.001$ ,  $p = 0.089$ ,  $p <0.001$ , respectively)(Fig. 9G). Interestingly, the aggression activity  
364 was also significantly higher at week 6 post injury in dark phase compared to the light  
365 phase ( $p = 0.009$ ) (Fig. 9G). These data would suggest TBI have an acute increase in  
366 aggression, which decreases with time at both light and dark phases.

367

## 368 **Assessment of injury severity**

### 369 *Behavioural assessment*

370 The BBB scores were measured daily during week 1 post injury, and then weekly up to  
371 12 weeks post-injury in SCI and non-CNS injured control animals. Baseline pre-surgery  
372 scores in both groups consisted of a BBB score of 21 (no functional impairments). The  
373 scores were sharply reduced in SCI animals immediately after surgery (values  $<4$  during  
374 the first week post-injury) indicating limited hindlimb movements following CNS injury (Fig  
375 9A). A subsequent gradual improvement was observed from week 3, reaching a plateau  
376 by week 7 post-injury. Non-CNS injured control animals showed no functional impairment  
377 after surgery, displaying baseline scores of 21 over the 12 weeks (Fig. 10A).

378 mNSS scores were measured in TBI animals for 3 days post injury. Following a normal  
379 baseline average score of 0 points one day prior to surgery, a mild functional deficit (2/20  
380 score) was detected on the first day post-injury, as expected for a mild "closed head" TBI  
381 model. (Fig. 10B).

382 Correlation tests showed a significant strong association between the automated RFID  
383 activity data recorded during the dark phases in the SCI groups and their BBB scores  
384 ( $R=0.08806$ ;  $P<0.001$ ) and a relatively good correlation during the light phases activity  
385 data recordings ( $R=0.5169$ ;  $P=0.001$ ) (Fig. 11A). (do we need to add per 5 min in the  
386 legend of the graph ?

387

### 388 *Histological assessment*

389 Gross histological analysis in the SCI group revealed elongated partial thickness spinal  
390 cord lesions, with significantly larger areas of cavitation associated with loss of CNS  
391 tissue surrounded by disordered tissue extending away from the lesion (Fig. 10C & E).  
392 The non-CNS injured control group exhibited no histological damage in the spinal cord  
393 (Fig. 10C & E).

394 Gross histological analysis in the TBI group revealed no significant morphological  
395 changes in tissue between contused brain and age-matched control brains (Fig 10D).  
396 However, a significant enlargement of the ventricles was observed when compared to the  
397 control group (Fig. 10D & F).

398 Correlation tests showed a strong association between the automated RFID activity data  
399 recorded during the dark phase and the cord cavity size ( $R=0.9755$ ,  $P=0.0123$ ; Fig.11C),  
400 in accordance with the correlation observed between BBB and cord cavity size  
401 ( $R=0.9297$ ,  $P=0.0358$ ; Fig.11B) in the SCI group. However this correlation was moderate  
402 when associated with the recorded activity during the light phase ( $R=0.7234$ .  $p=0.1495$ ;  
403 Fig 11C).

404 Correlations between the automated RFID activity data recorded during both the dark and  
405 the light phases and the ventricle size in the TBI group were moderate ( $R=0.5793$ ,  
406  $P=0.5261$  and  $R=0.5609$ ,  $P=0.2511$ ; respectively) (Fig. 11E), similar to that for the mNSS  
407 and the ventricle size ( $R=0.8137$ ;  $P=0.09$ ; Fig. 11D).

408

## 409 **Discussion**

410 The present study reports the ability to monitor spontaneous behavioural phenotype of  
411 rat models of SCI and TBI group-housed in their home cages during 12 weeks post-  
412 injury using an automated recording system. Distinct changes in phenotype within each  
413 injury group at specific time points after injury, and also differences between the injury  
414 groups were identified. SCI animals exhibited less locomotor activity during the acute  
415 period following injury. TBI animals exhibited heightened aggressive behaviour during the  
416 acute and mid-term period after injury. The automated home cage recording system  
417 successfully enabled the continuous acquisition of individual behavioural and  
418 temperature data from group-housed SCI, TBI and control rats, in their home cage  
419 environment. Such home cage approaches have great potential to improving the  
420 relevance of behavioural testing in such complex CNS injury models, facilitating long  
421 term, non-invasive, non-task driven assessment, and with minimal environmental  
422 interference.

423 Our findings also suggest that SCI has a significant impact on the animals' behaviour.  
424 Significant reductions in their locomotor and rearing activities were expected due to  
425 hindlimb paralysis, but its impact on grooming care was a novel observation. Undertaking

426 behavioural testing during the early phases post-injury can be challenging as it is likely to  
427 have more confounders associated to interventions such as surgery, anaesthesia and  
428 analgesia. But locomotor function tests are likely to show more significance during early  
429 injury times than later ones when the SCI animals have already started to regain  
430 locomotor and homeostasis functions and when improvements may be more difficult to  
431 assess. Therefore, assessing the spontaneous behaviour of SCI animals within their  
432 home cage environment provides a great source of very valuable new information with  
433 great potential for assessing the impact of any possible therapeutic approach on non-  
434 locomotor related behaviours in SCI animals. It also highlights the importance of providing  
435 a good care and welfare monitoring protocols supporting grouped housing conditions to  
436 enhance as much as possible the animals' natural behaviour, particularly within the early  
437 acute phase post-injury.

438 In this study, grooming activity was reduced in SCI animals, particularly during the first  
439 week post-injury, when compared to non-CNS injured control animals, possibly  
440 associated to injury-linked mechanical impairments. By week 6 and 12, SCI animals  
441 showed an improvement in grooming. It is important to note that our more detailed  
442 observations were carried out in time frames expressing high frequency of activity  
443 occurrence, thus we may be missing in grooming activity during more stationary  
444 behaviour periods. Alterations in grooming behaviour have been repeatedly studied in rat  
445 SCI, but mostly associated to the biomechanical impairments to groom effectively as a  
446 behavioural test, mostly in cervical SCI models (28-30). Grooming is also associated to  
447 the animal's self-care routine, and its failure may also be associated with mood  
448 impairments, such as depression caused by boredom and lack of social interaction (31).

449 Self-neglect and poor care has been reported in depressed SCI patients (32) and  
450 similarly, SCI injury has also been associated with the animal's depressed state (33). Our  
451 study provides an objective tool to investigate such socially associated cognitive  
452 impairments in grouped animals and through long term recordings. It is quite likely that  
453 prolonged immobility in SCI animals might affect their mood, triggering self-neglect and  
454 diminishing self-grooming behaviour, similar to that seen in humans with SCI (34).

455 Rearing activity was also reduced in SCI animals during the first week post injury,  
456 compared to week 12 post injury when spontaneous recovery in hindlimb functions have  
457 occurred. Rearing is irrefutably dependent upon hind limb function and thus linked to BBB  
458 scorings, and SCI animals have been shown to progressively regain function by 2-3  
459 weeks post-injury. Functional CNS deficits may improve by local neuroplastic changes  
460 (35) and also gradual strengthening of local signaling networks such as central pattern  
461 generators, as previously suggested in SCI models (36). Therefore, automated home  
462 cage recording system may facilitate new avenues to assess the pace and extent of  
463 recovering of rearing activity, and in particular, allow to investigate the role of housing  
464 enrichment to stimulate regular exercise and its impact on regaining functionality.

465 One of the major concerns when monitoring SCI and TBI animals is the ability of the motor  
466 and cognitively impaired animals to feed and drink. Our study demonstrated using our  
467 injury paradigm that neither TBI nor SCI significantly influenced feeding or drinking  
468 behaviour. Yet the ability of the injured animals to access food and water should not  
469 undermine the importance of good care and welfare monitoring of these animals, as  
470 maintenance of an appropriate schedule of feeding and drinking will also have a direct  
471 impact on the functional recovery following SCI and TBI (37).

472 We were also able to identify changes in aggressive behaviour between TBI, SCI and  
473 control animals. The data demonstrated an increase in night-time aggression at week 1  
474 post injury in TBI animals, compared to SCI and control animals. Such increased  
475 aggressive behaviour persisted by week 6 post-injury in TBI animals, but was no longer  
476 detected by week 12, when compared to SCI animals. Such assessment were based on  
477 individual behavioural patterns, as described in Fig. 2, and may associated to specific  
478 alteration of social rank rather than equal degree of aggression patterns for each  
479 individual animal. The effect of TBI on aggressive behaviour in rodent models has  
480 previously been reported in mice, but there are no reports in rat TBI (38). Assessment of  
481 aggression in laboratory rodents is intrinsically challenging, owing to the diversity of  
482 behavioural patterns and its multidimensional causes, expressions and functions. Animal  
483 studies on aggression tend to focus on the ethological relevance to survival; that is  
484 aggression that promotes access to food, territorial homing, mating, offspring protection  
485 or social rank. However, CNS injury may precipitate a pathological aggression that  
486 challenges such ethologically driven adaptive behaviour (39)- it is such maladaptive  
487 aggression that we have attempted to evaluate here. So the challenges are associated  
488 with the interpretation of different tests used for aggression, which are generally based  
489 on stimulating a defensive response, the lack of clear relationship between aggression,  
490 fear or defensiveness and how to account for the inhibitory effects of fear on the  
491 aggressive response. Furthermore, the lack of clear translation between categories of  
492 animal and human aggression, as human aggression is directly linked to complex  
493 societal perceptions (40). Most preclinical testing for aggression is carried out using the



494 tube dominance test (41), but it remains uncertain whether outcomes directly relate to  
495 human aggression.

496 The high incidence of aggressive behaviour in TBI patients is a major health concern (42,  
497 43). Translational strategies need to search for new avenues to understand and to  
498 evaluate aggression behaviour in animal models. The aggression data provided in this  
499 study, based on the individual observation of published behaviour patterns in housed-  
500 grouped animals, provides a new approach to monitor such challenging behaviours in  
501 SCI or TBI animals (44-46).

502 There are several challenges and limitations in this study. Firstly, our automated home  
503 cage recording systems were installed in our standard rat housing room, with no specific  
504 restrictions on access to the room by other staff. Therefore, there was no specific control  
505 for external stimuli influencing rats' activity (e.g. general daily husbandry activities, access  
506 to the room by other researchers). Our primary objective was to assess the feasibility of  
507 using the automated recording system in our animal unit, while maintaining our regular  
508 husbandry and our animal care procedures, and thus minimizing confounding effects due  
509 to stress or other environmental effects associated to changing the housing conditions of  
510 the tested animals. Yet, most of the disruptive periods could be easily identified by the IR  
511 video recordings and it was easy to exclude them from analysis based on time recordings.  
512 A possible solution would be to keep the automated recording system in a dedicated room  
513 with access restriction.

514 Secondly, although the generation of the body temperature and number of transition data  
515 take approximately 5 min to complete using the HCA software analysis, the revision of IR  
516 video recording is very time consuming. It may require multiple annotations when

517 assessing individual behaviour in group animals (each footage was reviewed 3 times to  
518 focus on each one of the 3 caged animals in each revision). However, by integrating the  
519 RFID and the IR video recording data we were able to rapidly select specific time frames  
520 associated with the automated RFID data. While this approach allowed us to investigate  
521 the detailed behaviours, including grooming, drinking, eating, aggression and rearing, our  
522 combine analysis was focused on the periods of high activity for each cage individually  
523 (expressed by  $\geq 1$  animal within the  $n=3$  animals housed per cage; see Suppl. Fig. S2)  
524 and that this was not the same time point for each cage. Therefore, each cage was  
525 analysed at different times of the day, rather than a continuous assessment of each  
526 individual animal per group. Data storage and handling could also be an issue, and it is  
527 mostly associated with the storage of the IR video recording data due to the large data  
528 files. However, as mentioned above, using the RFID automated data allows for a rapid  
529 selection of specific time frames of video recording, improving data storage and  
530 management.

531 Finally, we decided to use female rats for SCI studies and male rats for TBI studies as  
532 these are the most commonly used sexes for the models. Females rats are often preferred  
533 as easier to support bladder dysfunctions whilst male rats are driven by male TBI  
534 prevalence. Although our preliminary studies on naïve animals (non-injured,  $n=3$  males  
535 and  $n=3$  females, showed no differences on baselines activity and/or body temperatures  
536 (Fig. 3), it is possible beyond baseline data that we cannot exclude differential responses  
537 to CNS injury based on sex related endocrine effects.

538

539 *Impact on animal care and welfare and future perspectives*

540 The use of automated home cage analysis system has provided a unique opportunity for  
541 evaluating the spontaneous behaviour in individual grouped-housed SCI and TBI animals  
542 for a long 3 month period after injury. The system has facilitated the identification of novel  
543 behaviour insights in SCI and TBI rat models, such as transient increase in aggression  
544 following TBI, a transient reduction in grooming and rearing activity following SCI, and no  
545 effect of either TBI or SCI on drinking or feeding patterns. The provision of such a unique  
546 source of behavioural observations in SCI, TBI and control group-housed animals,  
547 acquired in their own environment and with minimal interference, represents a major  
548 improvement in the quality, quantity and scientific value of the experimental data  
549 generated per animal. The monitoring versatility of this automated system to assess  
550 cognitive /social behaviour in grouped animals compared to conventional out-of-cage  
551 tests carried out in single isolated animals may enables complementary avenues to  
552 identify socially-dependent behaviours that may be favourable or adversely affected by  
553 treatment intervention. This along with the ability to support long term studies, with 24/7  
554 recordings, may impact on the number of animals required for experimentation. Moreover,  
555 being able to continuously and accurately monitor behaviour and body temperature has  
556 significant implications for laboratory animal welfare; it can inform refinement of care and  
557 monitoring protocols, severity limits and humane endpoints (17), which is particularly  
558 pertinent for neurotrauma models. For instance, we report considerable impairments of  
559 locomotion and thermoregulation in SCI animals during the initial weeks post-surgery,  
560 which should translate in improved monitoring and care protocols. The ability to support  
561 such non-invasive long-term behaviour assessments in complex injury models while  
562 maintaining the animals housed in their own environment and cohorts represents an

563 important experimental refinement (in accordance with the 3Rs) and, by providing a  
564 valuable complementary approach to other conventional tests, may overall strengthen  
565 our understanding of the behaviour outcomes.

566 This technology considerably complements the accurate detection of subtle changes in  
567 behaviour phenotype of these complex CNS injury models. For example, handler-  
568 directed, compensatory aggression in response to removal from the home cage, for  
569 running a tube dominance test, may render increases in baseline aggression secondary  
570 to the neuroinjury undetectable (47). The automated analysis system provides an  
571 accurate comprehensive platform for investigating a wide range of behaviours, free of  
572 experimenter and environment interference. In summary, this technology represents a  
573 major advancement on current methods for studying behaviour in neurotrauma models,  
574 with great potential to enhance translational power of preclinical neurotrauma studies.  
575 This warrants its application in further neurotrauma and drug discovery research, in order  
576 to aid the development of effective new treatments for SCI and TBI.

577

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584 system in neurotrauma models.

585

## 586 Supporting information

587 **Supplementary Figure 1.** (A) The 21-point BBB (Basso, Beattie, Bresnahan) locomotor  
588 scale (6) was used to assess the hind limb recovery in rats following thoracic SCI, based  
589 upon observation of their spontaneous open-field locomotion. (B) The mNSS (modified  
590 neurological severity score) for TBI in rats was used. It was modified from the original  
591 score (48) to accommodate the mild nature of the closed head injury used in this study.

592

593 **Supplementary Figure 2:** Method of data sampling to assess in detail specific  
594 behavioural expressions (e.g. aggression, grooming, rearing, feeding and drinking) by  
595 reviewing the RFID digital data with the IR video recordings. (A) The objective was to  
596 elucidate during a representative day of per group per week, when the rats were most  
597 active and within that time frame which behaviours were being displayed. We selectively  
598 reviewed the periods of maximum activity as we hypothesised that these periods should  
599 show maximal expression of the stereotypical behaviours that characterise each  
600 phenotype. Notably, we plotted 'transitions' against time because the HCA system/report  
601 recorded 'transitions' as a proxy for 'activity'. Specifically, one transition defines the  
602 movement of an animal's RFID chip across the electromagnetic field boundary between  
603 two adjacent antennae. (B) A representative graph displaying the total number of  
604 transitions. Yellow and grey shaded areas indicated the light and dark phases,  
605 respectively. The arrows indicate the peaks with the greatest number of transitions  
606 occurring within a 5 min interval, per 3 h division of each 'light or dark' phase that are not

607 caused by external events (e.g. person entering the room, changing water  
608 bottle)(arrowheads).

609

610

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646 [analysis-and-](http://pilas.org.uk/automated-homecage-behavioural-analysis-and-the-implementation-of-the-three-rs-in-research-involving-mice) the-implementation-of-the-three-rs-in-research-involving-mice.

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729

## 730 **FIGURE LEGENDS**

731 **Figure 1. Overview of the experimental design.** After the baseline recording of the  
732 behavioural tests, animals were subjected to CNS injury and implanted with the RFID chip  
733 subcutaneously before they were returned to their group in their home standard IVC



734 cages. During automated analysis using the home cage analysis (HCA) unit, the ICV cage  
735 were secured directly above the baseplate RFID reader to derive the positional and  
736 temperature information for each individual animal from their RFID chip. An infrared HD  
737 video camera captured an infrared gray scale video, supported by the illumination of an  
738 array of infrared LEDs for the light/dark cycle recordings. RFID baseplate and video data  
739 (24/7 h for 3 days/week for 12 weeks) was captured in a mini-computer. HCA units were  
740 kept inside the rat housing room, to maintain environmental housing conditions.  
741 Functional assessments were carried out daily for the TBI animals (mNSS scores; up to  
742 3 days) and weekly for SCI and control animals (BBB scores; up to 12 weeks). 12 week  
743 post-injury animals were humanely killed and tissue fixed-perfused for histology.

744

745 **Figure 2. Definitions of the five behavioural expressions selected and analysed in**  
746 **detail in this study.** These were aggression, individual grooming, rearing, feeding and  
747 drinking, with images directly acquired from the IR video recordings.

748

749 **Figure 3. Locomotor activity and body temperature of naïve rats.** (A) Data displays  
750 the locomotor activity of the animals derived from the number of transitions detected by  
751 the baseplate RFID reader from the individually ID chipped group-housed rats. (B) No  
752 significant difference in locomotor activity was observed between naïve male and female  
753 rats and light and dark phases. (C) Data displays the body temperature recording of the  
754 animals measured through the subcutaneous chip in the lower flank of the animals. (D)  
755 No significant difference in subcutaneous body temperature was observed between naïve  
756 male and female rats, but significant difference was observed between light and dark

757 phases for both genders. Data plotted for male (blue) and female (red) rats over a 5 day  
758 period from 24/7 recordings; mean +/- SEM of 3 rats per group. The 12 h light-dark phase  
759 is indicated by white-black bars above graph.

760

761 **Figure 4. Locomotor activity in control, SCI and TBI animals at various weeks post**  
762 **injury.** Data display the locomotor activity (number of transitions automatically detected  
763 by the RFID reader) from the individually ID chipped group-housed rats. Representative  
764 data plotted over 2 days per week 1 (A-C), week 6 (D-F), and week 12 (G-I) post surgery  
765 from 24/7 recordings; mean +/- SEM per group. Note the lack of light/dark circadian  
766 pattern in SCI and TBI animals during the first week post injury compared to the control  
767 group. Furthermore, SCI group showed decreased activity patterns during the 1 week  
768 post injury. The 12 h light-dark cycle is indicated by white-black bars above graph.

769

770 **Figure 5. Comparison of locomotor activity in control, SCI and TBI between weeks**  
771 **post injury and injury groups.** (A) Significant decrease in locomotor activity in SCI group  
772 compared to the control and TBI group at week 1 post injury. Significant increase in  
773 locomotor activity observed in the dark phase compared to the light phase for all  
774 groups.(B) Significant increase in locomotor activity in SCI group compared to the control  
775 and TBI group at week 6 post injury. Significant increase in locomotor activity observed  
776 in the dark phase compared to the light phase for all groups. (C) At week 12 post injury,  
777 no difference between injury groups, but significant increase in locomotor activity in the  
778 dark phase compared to the light phase for all groups were observed. (D) Temporal  
779 changes in locomotor activity were observed in SCI animals within the light or dark phase.

780 (E) No temporal changes in locomotor activity was observed in TBI animals. (F) Temporal  
781 changes in locomotor activity were observed in non-CNS injured control animals within  
782 the light or dark phase. The 12 h light-dark cycle is indicated by white-black bar above  
783 graph.

784

785 **Figure 6. Body temperature in control, SCI and TBI animals at various weeks post**  
786 **injury.** Data display the subcutaneous body temperature (automatically detected by the  
787 RFID reader) from the individually ID chipped group-housed rats. Representative data  
788 plotted over 2 days per week 1 (A-C), week 6 (D-F), and week 12 (G-I) post surgery from  
789 24/7 recordings; mean +/- SEM per group. Note the slower ability of SCI and non-CNS  
790 injured control animals to recover normothermia immediately after surgery, compared to  
791 the TBI groups even when warm post-surgery recovery chambers were used. Body  
792 temperature levels did not show a circadian light/dark cycle pattern during the first week  
793 post-surgery in all groups. The 12 h light-dark cycle is indicated by white-black bars above  
794 graph.

795

796 **Figure 7. Comparison of body temperature in control, SCI and TBI between weeks**  
797 **post injury and injury groups.** (A) No significant difference in body temperature  
798 between the groups at week 1 post injury. (B) Significant decrease in body temperature  
799 in SCI and TBI group at light phase compared to the dark phase at week 6 post injury.  
800 Significant increase in locomotor activity observed in the dark phase compared to the light  
801 phase for all groups. (C) At week 12 post injury, no significant difference between injury  
802 groups. (D) Temporal changes in body temperature were observed in SCI animals within

803 the light and/or dark phase. (E) Temporal changes in body temperature was observed in  
804 TBI animals within the light and/or dark phase. (F) No temporal changes in body  
805 temperature were observed in non-CNS injured control animals. The 12 h light-dark cycle  
806 is indicated by white-black bar above graph.

807

808 **Figure 8. Assessment of manually selected behavioural expressions for feeding,**  
809 **drinking, grooming and rearing between control, SCI and TBI animals.** (A-C) No  
810 significant difference was observed in feeding between all groups at week 1, 6 and 12  
811 post injury. (D-F) No significant difference was observed in drinking between all groups  
812 at week 1, 6 and 12 post injury. (G-I) SCI animals showed a decreased grooming activity  
813 at week 1 during dark phase compared to control group ( $P=0.04$ ), but by week 6 and 12,  
814 no significant difference was observed between the groups. (J-L) No significant difference  
815 was observed in rearing between all groups at week 1, 6 and 12 post injury. The  
816 expression of a given behaviour was calculated as the duration of time (sec) that each  
817 behaviour was performed by at least 1 animal within the cage during the 5 min period of  
818 observation. Data presented as mean  $\pm$  SEM of 6 animal per group and during the light  
819 and dark phases. The 12 h light-dark cycle is indicated by white-black bar above graph.

820

821 **Figure 9. Assessment of manually selected behavioural expressions for temporal**  
822 **rearing within groups and aggression between and within groups.** (A) No significant  
823 difference was observed in temporal rearing within the non-CNS injured control groups at  
824 week 1, 6 and 12 post injury. (B) Significant difference was observed in rearing between  
825 week 1 and week 12 at dark phase in SCI animals. (C) No significant difference was

826 observed in temporal rearing within the TBI groups at week 1, 6 and 12 post injury. The  
827 expression of a given behaviour was calculated as the duration of time (sec) that each  
828 behaviour was performed by at least 1 animal within the cage during the 5 min period of  
829 observation. Data are presented as mean +/- SEM of 6 animals per group and during the  
830 light and dark phases. White bars indicate LAM group, gray bars indicate SCI group, and  
831 black bars indicate TBI group. The 12 h light-dark cycle is indicated by white-black bar  
832 above graph.

833

834 **Figure 10. Assessment of injury severity using conventional behavioural and**  
835 **histological analysis.** (A) BBB score of non-CNS injured control (blue square) and SCI  
836 (black diamond) animals for 12 weeks post-surgery displayed severe initial hindlimb  
837 impairment followed by some spontaneous functional improvement by week 6 post injury  
838 compared to control animals. (B) mNSS score for the closed TBI injury (black circle) for 3  
839 days post-surgery displayed limited functional impairment 24 h post compared to control  
840 animals (blue square). (C) Representative Cresyl violet (Nissl) staining of serial horizontal  
841 sections of spinal cord from control (left) and SCI (right) showing the degree of injury and  
842 tissue damage across the whole spinal cord in SCI animals compared to the control  
843 animals at 12 weeks post-surgery. (D) Representative Cresyl violet (Nissl) staining of  
844 serial coronal sections of brain from closed TBI (right) displayed ventriculomegaly  
845 compared to control brain (left) at 12 weeks post-surgery. (E) Analysis of the contused  
846 spinal cord revealed significantly larger cavity than the control spinal cord. (F) Analysis  
847 of the brain revealed significantly larger ventricles in the mild traumatic brain injury than

848 the control brain. \*  $p < 0.05$  and \*\*\*  $p < 0.001$ , Student's *t* test. Scale bars panel C, 0.5 mm  
849 and panel D, 1 mm.

850

851 Figure 11. Correlation analysis between the automated RFID activity data and  
852 conventional behaviour and histological tests. A) Activity data (nm of transitions) shows  
853 highly significant good positive relationship with the BBB scores in the SCI group during  
854 the dark phases and light phases of recordings across the 1, 6 and 12 weeks post-injury.  
855 B) Good negative correlation between the BBB scores and cord cavity size ( $\text{mm}^2$ ) in the  
856 SCI group. C) Good negative relationship between the RFID activity data recorded during  
857 dark phases and the cord cavity, and moderate relationship for the light phase data. D)  
858 Moderate correlation between the mNSS and the ventricle size and E) the RFID activity  
859 data recorded in the dark and light phases and the ventricle size in the TBI group. (should  
860 you change the dark and light colour in the graph? Also the signs on Fig11A not too clear  
861 on grey scale..)

862