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Abstract:

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

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

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

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

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

Introduction

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

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

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

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

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

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

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

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

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

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

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

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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

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

Methods

Ethical statement

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

Animal housing and husbandry

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

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

SCI and TBI surgical procedures and in vivo experimental design

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

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

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

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

Conventional behaviour tests

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

Histology

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

Automated home cage recording system

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

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

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

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

Data acquisition

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

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

Data sampling and analysis

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

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

Statistical analyses

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

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

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

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

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

Results

Subacute behavioural analysis of naïve animals

Locomotor activity and temperature data demonstrating circadian pattern

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

Locomotor activity changes in SCI, TBI and Control animals

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

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

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

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

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

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

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

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

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

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

Rearing behaviour activity increases over time after SCI

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

Aggression was significantly higher in TBI animals early after injury

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

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

Assessment of injury severity

Behavioural assessment

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

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

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

Histological assessment

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Impact on animal care and welfare and future perspectives

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

Supporting information

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

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

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

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FIGURE LEGENDS

Figure 1. Overview of the experimental design. After the baseline recording of the behavioural tests, animals were subjected to CNS injury and implanted with the RFID chip 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

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

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

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

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

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

Figure 7. **Comparison of body temperature in control, SCI and TBI between weeks post injury and injury groups.** (A) No significant difference in body temperature between the groups at week 1 post injury. (B) Significant decrease in body temperature in SCI and TBI group at light phase compared to the dark phase at week 6 post injury. Significant increase in locomotor activity observed in the dark phase compared to the light phase for all groups. (C) At week 12 post injury, no significant difference between injury 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 +/- 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 12at dark phase in SCI animals. (C) No significant difference was

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

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