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PII: S0165-0270(19)30156-6
DOI: https://doi.org/10.1016/j.jneumeth.2019.05.015
Reference: NSM 8306

To appear in: *Journal of Neuroscience Methods*

Received date: 12 February 2019
Revised date: 13 May 2019
Accepted date: 28 May 2019

Please cite this article as: Schuch CP, Balbinot G, Jeffers MS, McDonald MW, Dykes A, Kuhl LM, Corbett D, An RFID-based activity tracking system to monitor individual rodent behavior in environmental enrichment: Implications for post-stroke cognitive recovery, *Journal of Neuroscience Methods* (2019), https://doi.org/10.1016/j.jneumeth.2019.05.015

Title: An RFID-based activity tracking system to monitor individual rodent behavior in environmental enrichment: Implications for post-stroke cognitive recovery

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Submission Type: Research Article

Highlights:

- We used a low-cost system for monitoring individual rat activities in home cage
- Prefrontal cortex damage resulted in behavioural flexibility deficits
- RFID tracking is a feasible tool to predict post-stroke impairment and recovery

Abstract

Background: Diminished cognitive flexibility is a common form of executive dysfunction that results from stroke in the prefrontal cortex. Potential therapies targeting this type of cognitive deficit following stroke are lacking.

New Method: Here, we used environmental enrichment (EE) as a rehabilitation approach, integrated with a radio frequency identification (RFID)-based activity tracking system to evaluate the contribution of individual EE elements to promote cognitive recovery. Male and female Sprague–Dawley rats received either sham surgery or endothelin-1 (ET-1) induced focal ischemia targeting the medial prefrontal cortex (mPFC). Cognitive flexibility was assessed through an egocentric-spatial version of the Morris Water Maze (MWM) task.

Results: Prefrontal cortex damage resulted in impaired reversal learning using the egocentric MWM and reduced physical activity in the running wheel, while social interaction was not affected. EE exposure (2 hours/day, 5 days/week, for 5 weeks) improved cognitive flexibility in reversal learning of egocentric MWM for both stroke and sham rats.

Comparison with existing method: As changes in cognition post-stroke can be subtle and difficult to detect using conventional behavioural assessment, we suggest that the implementation of individualized automated animal tracking as used herein will ultimately help decipher whether individual components of EE are important for promoting cognitive recovery post-stroke. Conclusion: This study represents an attempt to better align preclinical and clinical implementations of EE and facilitate the uptake of this intervention in the clinical setting.

Key words: Medial Prefronatal Cortex; Stroke; Stroke Recovery; Cognitive Flexibility; RFID; Tracking; Morris Water Maze; Environmental Enrichment; Behaviour

1. Introduction

Cognitive impairments are prevalent following clinical stroke with outcomes ranging from mild cognitive impairment to dementia[1–4]. It has been estimated that about two-thirds of stroke survivors experience some form of cognitive impairment or decline[3,5]. The most commonly observed cognitive impairments in stroke patients are reduced mental speed, spatial

neglect, attention and memory deficits, and executive function disorders[3]. Deficits in executive function, mediated by the prefrontal cortex, include working memory, goal-based decision making, learning, and proper application of rules[6]. Despite subtle onset, cognitive impairment post-stroke may evolve to dementia[7] and has been associated with a three-fold increase in risk for mortality[8]. Consequently, novel therapies designed to restore cognitive function following stroke are imperative.

Despite the need for preclinical stroke models to better model the human condition[9], few preclinical stroke models target higher-order cognitive dysfunction[4,6,10]. We[1,2] and others[11–13] have previously shown that the prefrontal cortex of the rat is involved in a complex array of cognitive processes and functions. The medial prefrontal cortex (mPFC) in rodents is essential for set-shifting behavior, which requires the cognitive flexibility to shift between responses, perceptual dimensions, and rules[1,13]. Both the prelimbic (PL) and infralimbic (IL) regions of the rat mPFC contribute to this ability. Rats trained to use one cognitive strategy have difficulty learning a new strategy if the PL/IL is injured[14]. Thus, the PL/IL is important for learning new tasks in place of old ones, but it may also be required to switch between familiar tasks[15]. It has also been shown that mPFC damage can disrupt Morris water maze (MWM) navigation strategies[16–18]. While the hippocampus is involved in allocentric based place-learning (using distal cues independent of body orientation), the prefrontal cortex plays a significant role in acquisition of spatial tasks requiring egocentric orientation (internal cues, directional heading, and proximal cues)[19–21]. In other words, while the allocentric spatial strategy depends on external cues to locate a target in space, egocentric navigation relies on internal cues and repeated use of relatively fixed motor movements to locate the target[22–24].

While single pharmacological and nonpharmacological approaches have been utilized for patients with prefrontal cortex stroke[25], multiple target interventions, such as combinations of physical and cognitive stimulation, appear more advantageous to augment endogenous brain plasticity processes and enhance functional recovery[26,27]. Indeed, environmental enrichment (EE) has been extensively used to treat several nervous system disorders, including stroke[28–30]. EE stimulates experience-dependent plasticity through a combination of factors such as social interaction, exercise, and nonspecific sensory, motor, and cognitive stimulation[31]. As expected, this multifaceted intervention activates a cascade of molecular pathways which are potentially beneficial for stroke recovery[32,33]. A recent review from our group[34] highlighted that preclinical

EE improves both motor and cognitive function following stroke. Nevertheless, the broader range implementation of EE into the clinical domain is challenging and requires an alignment between preclinical and clinical features of EE. For human stroke survivors, the intensity of training and the level of motivation for a particular task are known to play important roles for motor recovery[35]. Recently[36,37], wearable sensor systems have been used to better understand the importance of the dose of free-activities (outside rehabilitation service) on motor performance and recovery. These studies suggest that this ecological approach - sensor-based motor performance assessment - provides a more accurate outcome than self-reported activities. Similarly, some preclinical studies have monitored EE activities to investigate the effects of the intensity of self-training on motor skill acquisition under normal and pathological conditions[38–40]. Conversely, after prefrontal focal ischemia, which results in subtle cognitive deficits, the influence of spontaneous "free-activity" on cognitive function has not been assessed. Monitoring individual home cage activities may be useful to characterize those most likely to respond to a given intervention[29,41] and consequently to detect novel predictors of cognitive impairment and/or recovery.

Here, following mPFC stroke we monitored rodent activity levels during EE rehabilitation using radio frequency identification (RFID) and also assessed performance on egocentric orientation in the MWM task. We hypothesized that measurement of the animals' natural behavior in EE using this technology might eventually be used to determine if individual components of EE (e.g. socialization, physical activity) are important predictors of subsequent post-stroke cognitive performance. Alternatively, the benefits conveyed by EE on cognition, like motor recovery, may instead depend on the multi-factorial nature of EE rather than an individual component[34,42].

2. Materials and Methods

2.1 Subjects and experimental timeline

Adult male and female Sprague–Dawley rats (200-250g) were purchased from Charles River Laboratories (Montreal, Canada) and kept on a 12 hour reverse light/dark cycle with food and water freely available. All procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the University of Ottawa Animal Care Committee. Prior to surgery and allocation into respective housing conditions, animals were

handled for one week. Animals were randomly assigned to four experimental groups: sham maintained in a standard environment (Sham SE: n=4 males and n= 4 females), sham exposed to EE (Sham EE: n=3 males and n=4 females), mPFC stroke maintained in SE (mPFC SE: n=3 males and n=4 females) and mPFC stroke exposed to EE (mPFC EE: n=4 males and n=3 females). Two female animals from the stroke group died during surgery (1 SE and 1 EE). Animals were pair housed for the duration of the experiment, except during EE, where rats not exposed to the EE where kept alone in a standard home cage. EE animals were implanted with two subcutaneous RFID tags to track their activities. Rehabilitation involving EE began one week after surgery. Following 2 weeks of exposure to EE, cognitive testing was performed. A detailed timeline of the experiment is presented in Figure 1a.

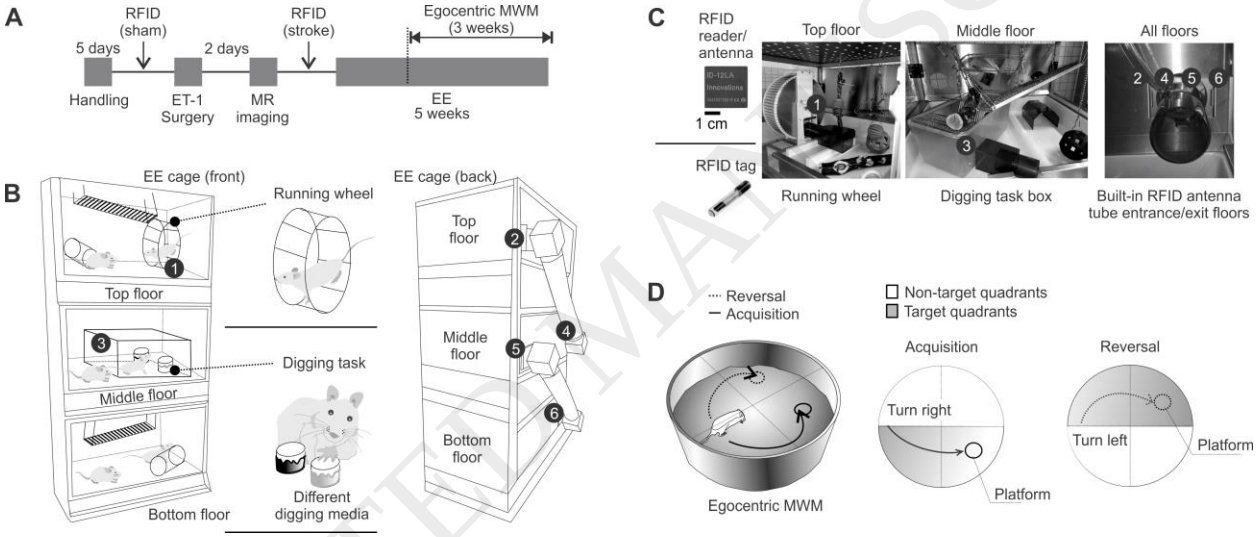

Figure 1: Experimental timeline, enrichment cage and MWM task. (a) Following arrival at the facility, animals were handled for one week. Sham animals assigned to EE groups were subcutaneously implanted with RFID tags on the same day as mPFC stroke surgeries. 48 hours after surgery, all stroke animals received MRI imaging and those assigned to EE groups were subcutaneously implanted with two RFID tags. (b) The following week, rehab animals were exposed to three-tiered EE cages with a total of 6 RFID sensors installed throughout the cage to monitor individual rat activity 2 hours/day, 5 days/week. (left) Sensor #1 monitored entrances into the running wheel and Sensor #3 into the novel digging task area. (right) Sensors #2-#6 monitored entrances and exits from each floor. (c) RFID reader/antenna, RFID tag and photographs of the instrumented EE cage setup. (d) At week 3 post-surgery cognitive testing began, wherein rats were trained in the MWM to learn an egocentric navigational strategy (submersed platform on the right) followed by a probe test. The start location was changed between each MWM trial so that rats had to use their own body position (egocentric) as the cue to the escape position of the maze and could not use external room cues (allocentric). Subsequently, a reversal period ensued, where the rats were trained for 5 days to locate the platform in the opposite side (on the left) followed by a probe test. This reversal challenges the

cognitive flexibility of the animals by requiring suppression of the old rule (turn right) and learning the new one (turn left). Animals were euthanized 5 weeks post-surgery.

2.2 Surgical procedures

Ischemic mPFC stroke was induced using endothelin-1 (ET-1) as described previously[1,2]. For all surgical procedures, animals were anesthetized with isoflurane (4.0% induction, 2.5% maintenance in 100% O2). Briefly, animals were placed in a stereotaxic frame and bilateral injections of ET-1 (0.8 µl, 400 pmol/µl in sterile water, Abcam, ab120471) were infused into four sites of the mPFC at the following Bregma coordinates: [AP + 3.5, ML ± 0.6, DV −5.2] and [AP + 2.5, ML ± 0.6, DV −5.0]. Upon completion of the surgery, rats were kept in a 37°C incubator until awake and were then returned to their home cages. Sham-operated animals were submitted to similar surgical preparation procedures as stroke animals, except no burr holes were drilled in the skull. The experimenter was blind to surgical group for all following procedures.

2.3 RFID implantation

Two days after mPFC stroke surgery and right after MRI imaging, stroke-EE rats were implanted subcutaneously with two RFID tags (Glass Capsule 125kHz, SEN-09416, SparkFun Electronics® - Niwot, Colorado) one between the shoulder blades and the second in the lower lumbar region. Sham-operated EE animals were also implanted with two RFID tags on the same day that animals were submitted to mPFC stroke. Each rat had a unique 32-bit ID code for each tag. A valid detection was considered only when both RFID tags were scanned in sequence as described elsewhere[38,39]. Rats were anesthetized during RFID tag implantation with 1.5%-2% isoflurane. RFID tags remained in place until the end of experiment.

2.4 Magnetic Resonance Imaging (MRI)

MR imaging was performed with a small animal magnetic resonance scanner (Agilent MR901 7T, General Electric ®, USA). 48 hours following stroke, rats were anesthetized (2.5% isoflurane) and restrained on an MRI bed. Structural images were acquired with a T2-weighted fast spin echo pulse sequence using the following parameters: 19 coronal slices; slice thickness = 800 microns; in-plane resolution = 78 microns; echo train length = 8; echo time = 27 ms; scan time = 5 minutes. Lesion volumes were quantified based on MR images using ImageJ® software (NIH, USA). In each slice, any observable hyperintensity in the stroke region was manually

traced, and the area of this region was measured. Infarct volume was then calculated by multiplying the sum of the infarcted area of each slice by the MRI slice thickness (800 microns).

2.5 Environmental enrichment

Starting one week after stroke, rehabilitation animals were given access to EE for 5 weeks, 5 days/week, 2 hours/day, in same-sex groups of 6 or 7, while those in non-EE groups were kept in standard cages. We chose 2 hours per day of EE, instead of 24 hour housing, to better align the translational aspect of EE with the clinical setting in which patients have limited time periods to engage in different physical and cognitive activities of their choice[9,34,43]. EE consisted of three-tiered ferret cages (LxWxH: 870x740x130 mm, Tecniplast®, Canada) that were interconnected by two tubes to increase floor space and allocate different tasks and activities to each floor (Figure 1b,c). The top floor contained the physical activity zone with a running wheel, ladders, beams and ramps. The middle floor contained a Plexiglas box that housed two small ceramic containers that were filled with different digging media, one of which contained a food reward. Both the digging media and type of food reward were changed weekly to maintain novelty. The bottom floor contained a variety of toys that were changed and reorganized weekly. Importantly, the regular introduction of novel objects and rearranging the EE cage configuration was intended to provide a cognitively stimulating environment[26]. Moreover, at the beginning of each EE session rats were placed on different floors to avoid any place preference.

To track RFID-implanted animals, a total of 6 antennae (RFID Reader ID-12LA, 125kHz, SEN-11827, SparkFun Electronics® - Niwot, Colorado) were installed in the EE cage to monitor the traversal of each individual rat between cage levels. Each antenna was able to read the RFID tags at a maximum rate of 125 Hz. Connections between different floors (antennae #2, #4, #5, #6), the entrance to the running wheel (antenna #1), as well the entrance to the regular housing box (antenna #3) had Plexiglas tubes with an antenna attached at the end to determine when the rat was moving through the tube.

The RFID reader (antenna) was run and controlled by a Raspberry Pi 3 microcomputer (Raspberry Pi Foundation, Cambridge, UK) connected to each EE cage. RFID reader operation and data logging were coded in Python v.3.6 (Supplementary material 1). CSV files containing the date, hour, minute, second, antenna ID and RFID tag number of each RFID scan event were

logged for each EE session, allowing monitoring of an individual rat's activity from 9am to 11am (dark cycle).

Rats exposed to EE had the opportunity to freely explore and engage in self-training on the activities available (wheel running, food reward grasping, beam, ladders, ramps, etc) as they desired. At the end of each EE session, the researcher exported the CSV log files containing all rat tracking data for the day and then returned each rat to their non-enriched pair-housing mate. LabVIEW® 8.5 custom software routines were developed to analyze the data (Supplementary material 2). Sequence readings through the combination of 2 antennae were used to define each floor zone: #2 and #4 was related to entrance/exit of top floor, #4 and #5 was related to middle floor, #5 and #6 was related to entrance/exit of bottom floor (Figure 1b). By analyzing the time of last detection for each rat at a given RFID sensor, we could estimate the time spent per floor and infer the type of activity in which each animal was mostly engaged. We also calculated a social interaction index that consisted of when 2 or more rats were together at the same floor, based on time stamp data and rat ID code.

During data processing we detected two error sources in the RFID tracking system, false readings due to rats lingering around the RFID sensor, and secondly, the spatiotemporal resolution of the RFID technology was too low to detect high-speed movements[44,45]. As previously mentionated, we considered a full passage only when both implanted RFIDs were detected in sequence. Thus, we were able to count correct and incorrect reading detections. After data processing we used a cut-off point, where detection errors up to 20% were excluded. Only male animals were included in the final EE data analysis. Female rats proved to be smaller and faster than males and our RFID-based tracking system did not consistently scan both tags of the female rats, and occasionally failed to detect the fast movements of female rats past the sensors.

2.6 Morris Water Maze test – Egocentric navigation

2.6.1 Apparatus:

To encourage use of an egocentric navigation strategy, a white curtain was hung from the ceiling to the upper edge of the Morris Water Maze and the release/goal position of the animal was changed between each trial (to prevent rats from utilizing extra-maze visual cues to locate the escape platform)[21].

We used a circular water tank measuring 1.85 m in diameter (45 cm deep; 24°C), containing a hidden escape platform (10 cm diameter) 2 cm below the surface of the water. Water was made opaque using non-toxic blue tempera paint (Colourations Simply Washable Tempera, 1:010883 BLUE). Four points along the circumference of the water tank were arbitrarily designated North (N), South (S), East (E), and West (W), thus dividing the maze into four zones. The platform was positioned in the middle of one of the following zones: SE, SW, NW and NE. We used Ethovision XT® software (v.12; Noldus Information Technology, Netherlands) to analyze the following parameters of the rats' path during each trial: escape latency (s), path length (cm), percentage of time spent in the target and non-target zones (%).

2.6.2 Behavioral procedures:

Acquisition: To explore the egocentric strategy, both the release site and the platform position varied from trial to trial, where the platform was always placed at a fixed distance to the right of the release site[18,22] (Figure 1d). Animals were given 4 trials per day, each trial lasted for a maximum of 90 seconds. Once on the platform, rats were allowed to rest for 10 seconds. If an animal did not locate the platform in 90 seconds, the experimenter guided them there and allowed the rats to rest on the platform for 30 seconds. Following this period, the animal was removed and placed in a holding bucket near the pool for a 30 second inter-trial interval. During this period the position of the platform was changed.

The training was initiated 3 weeks after the animals had been subjected to rehab (EE) and continued until an asymptotic performance was reached, or a maximum of 20 sessions were given to each animal. Asymptotic performance level was defined as an escape latency between 10-12 seconds on at least three trials out of four within a given session[18,24,46]. Once an animal reached asymptotic performance, it was exposed to the no-platform test (probe acquisition). The remaining animals kept training until criterion was reached or they completed 20 sessions[18].

Probe acquisition: A single 60-second no-platform trial was given 24 hours following asymptotic performance (from a randomly assigned start location). This test assessed reference memory for the most recent training session[46]. After the probe test, two additional daily sessions were given with the same procedures as during the initial task acquisition. This reinforcement of the learned strategy was provided to reduce confounding effects caused by the no-platform condition.

Reversal learning: following these two additional sessions, reversal testing was performed in the same way as an acquisition trial; however, the escape platform was placed at a fixed distance to the left of the release site[18]. This test assessed the flexibility of animals to extinguish their initial learning of the platform's position and acquire a direct path to the new goal position[46]. Reversal learning took place over 5 days.

Reversal Probe: A probe test was performed as before, 24 hours following completion of the reversal learning. Here we evaluated the retention of the new versus previously learned strategy[14,47].

2.7 Statistical analysis

MWM performance and EE data were analyzed using two-way repeated measures ANOVA followed by post-hoc tests for multiple comparisons, where appropriate. Acquisition vs reversal difference was analyzed using one-way ANOVA. Sidak-correction was used for correction of multiple comparisons of all ANOVAs. Pearson correlation was used to test for correlation between MRI lesion volume-impairment and several variables. Statistical analyses were performed using SPSS (v.24; IBM Corporation, USA). Statistical significance was set at $p < 0.05$. Values are expressed as mean ± standard error of the mean (SEM). Since both male and female rats were included in this experiment, sex was initially included as an independent variable in all analyses. However, as no significant effects of sex were observed in any analyses this variable is not included in further discussion for simplicity of interpretation.

3. Results

3.1 Stroke compromises physical activity engagement but not social interaction

MR imaging showed that mPFC stroke resulted in bilateral damage typically affecting the PL, IL and cingulate cortices, with an average volume of ~25.0 mm^3 (Figure 2a, b).

Overall, animals spent the majority of EE sessions on the middle floor (53.6%±3.00% stroke and 53.5%±2.3% sham), followed by the bottom (32%±3.46% stroke and 24%±2.34% sham), and top floors (13.8%±2.0% stroke and 21.6%±2.5% sham), respectively. ANOVA indicated that stroke rats spent a lower proportion of their time on the top floor ($F_{surgery\ (1,124)} = 5.951$; $p = 0.016$) compared to sham. Sham rats spent a lower proportion of their time on the bottom floor ($F_{surgery\ (1,124)} = 4.241$; $p = 0.042$, Figure 2c) compared to stroke. Across 5 weeks of

EE intervention there was no time effect for percentage of time spent on each floor between groups (Figure 2 d, e, f), except for week 5, where stroke rats spent a greater proportion of time on the bottom floor compared to sham ($F_{surgery\ (1,7)} = 4.653$; $p = 0.048$, Figure 2f). The top floor was the location of the running wheel, and the reduced time stroke animals spent on the top floor also corresponded to a lower number of entries to the wheel compared to shams ($F_{surgery\ (1,124)} = 5.601$; $p = 0.019$, Figure 2g). ANOVA revealed a significant time effect in the number of entries to the wheel ($F_{week\ (4,7)} = 9.843$; $p = 0.001$, Figure 2h) without differences between groups across time. A time effect was also observed in the number of entries to the digging task area ($F_{week\ (4,7)} = 8.773$; $p = 0.001$, Figure 2i) on the middle floor and for the social interaction index ($F_{week\ (4,7)} = 5.474$; $p = 0.010$, Figure 2j). Despite regular introduction of novel objects and/or rearranging the EE cage configuration there was a reduction of physical, cognitive and social engagement across the 5 week time period.

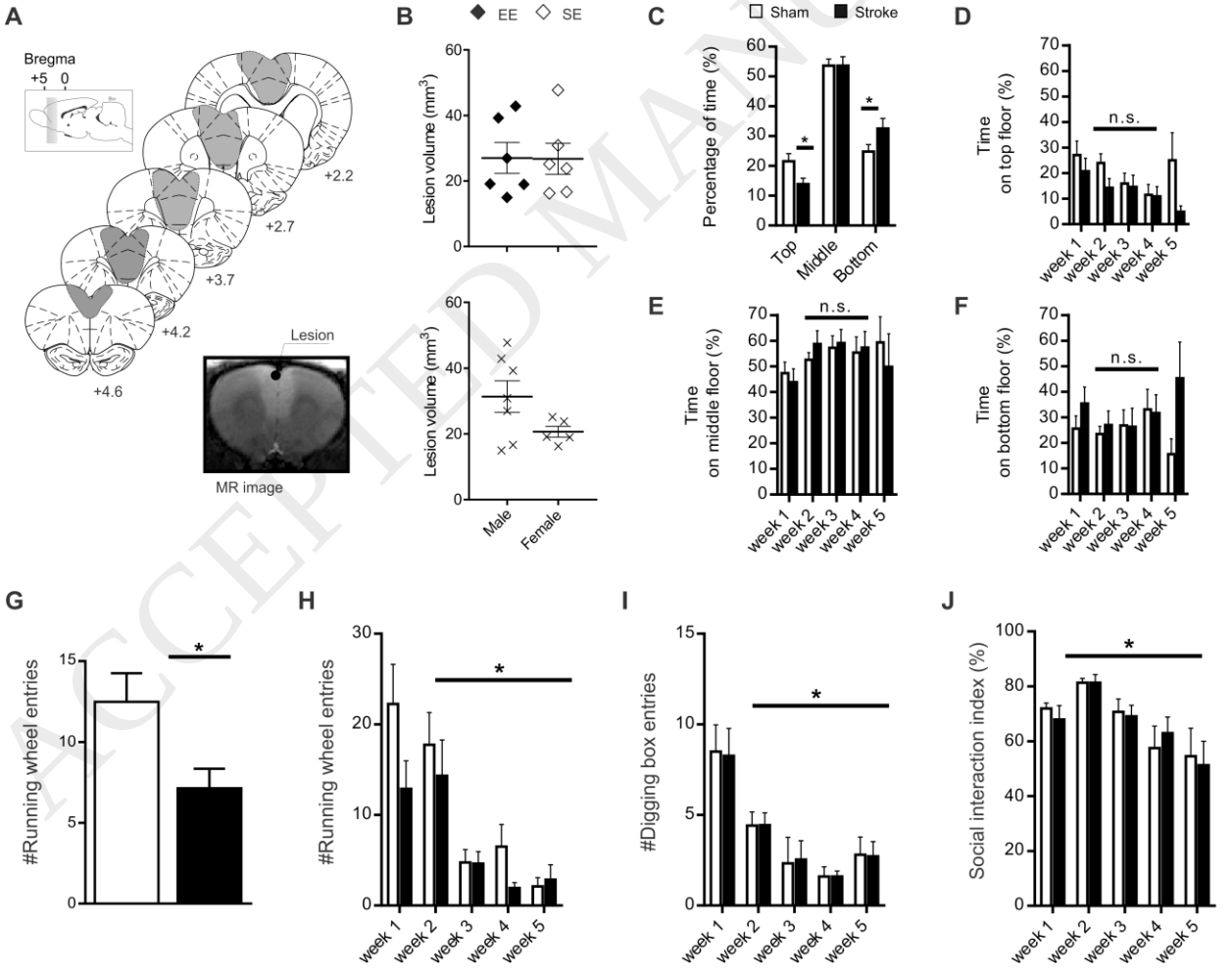

Figure 2: Image of lesion, infarct volume assessment and RFID-based tracking of enriched housing rats. (a) MRI image depicts a representative ischemic lesion; the lighter colored area in each coronal section is the ischemic region (light grey). (b) Lesion volume of individual animals by housing and sex (top: n=6 EE, n=6 SE; bottom: n=7 males, n=5 females). (c) On average, there was a significant surgery effect ($p < 0.05$) of percentage of time spent on top and bottom floors. (d,e,f) There was no time effect for proportion of time spent on top, middle or bottom floor. (g) On average, stroke rats showed lower number of running wheel entries compared to sham ($p < 0.05$) on the top floor. (h,i,j) There was a significant time effect in the the number of entries to the wheel and digging box and the percentage of social interaction index ($p < 0.05$) without differences between groups. Data are expressed as mean ± SEM; N.S. = not significant. In panels c-j only male animals were included (Stroke EE n=4, Sham EE n=3).

3.2 Environmental enrichment improves cognitive flexibility in reversal learning of egocentric navigation strategy

Across 17 days of acquisition and 5 days of reversal learning (Figure 3a) stroke and sham presented similar escape latencies in the egocentric MWM task (P>0.05). However, during the 5 days of reversal learning we observed a significant surgery effect ($F_{surgery\ (1,27)} = 4.400$; $p = 0.05$) on time spent in the target quadrant. Despite reaching the escape location in the same amount of time, mPFC stroke rats spent a lower proportion of trials in the target quadrant compared to sham ($p = 0.05$; Fig. 3b) after reversal of the egocentric orientated platform. This suggested that following the reversal of the learned strategy to escape the MWM, rats with mPFC stroke continued to use the former, less-direct, path to solve the maze, indicative of cognitive inflexibility. Lesion volume showed positive correlations with path length ($r = 0.582$, $p = 0.047$) and escape latency ($r = 0.665$, $p = 0.018$) on MWM performance (supplementary Table 1).

Probe trials were also analysed through time spent in the target quadrant, a two-way repeated measures ANOVA revealed a significant time effect ($F_{time\ (1,27)} = 26.365$; $p < 0.01$) across acquisition and reversal time points (Figure 3c). Post-hoc comparisons showed that animals spent more time in the target quadrants compared to the non-target quadrants during acquisition ($p < 0.01$) and more time in the non-target quadrants compared to target quadrants in the reversal ($p < 0.01$). There was also a reduction in time spent in the target quadrants between acquisition and reversal ($p < 0.01$). Followed by an increase in time spent in the non-target quadrants between acquisition and reversal ($p < 0.01$). Although not statistically significant (time by surgery interaction, $F_{time*surgery\ (1,27)} = 3.552$; $p = 0.075$), post-hoc revealed that stroke animals spent more time in the non-target zones using the previous learned egocentric strategy (target quadrant to the right) than the new strategy (to the left) in the reversal probe ($p < 0.01$).

Enriched animals' performance on egocentric navigation in the MWM test indicated a significant time by housing interaction on the path length ($F_{time*housing\ (1,27)} = 5.621$; $p < 0.05$) and escape latency ($F_{time*housing\ (1,27)} = 4.507$; $p < 0.05$) across acquisition and reversal time points (Figure 3d and g). There was no significant difference in path length or escape latency between enriched and non-enriched groups at either acquisition or reversal when these time points were considered separately (Figure 3e and h; $p > 0.05$). However, by transforming the data to the mean difference between acquisition and reversal we observed that for both path length ($F_{timediff(1,27)} = 5.621$; $p = 0.028$, Figure 3f) and escape latency ($F_{timediff(1,27)} = 4.507$; $p = 0.047$, Figure 3i) EE resulted in improved completion of the maze relative to sham between acquisition and reversal time points. Therefore, non-enriched animals showed impairment in cognitive flexibility when switching to the egocentric strategy compared to enriched animals.

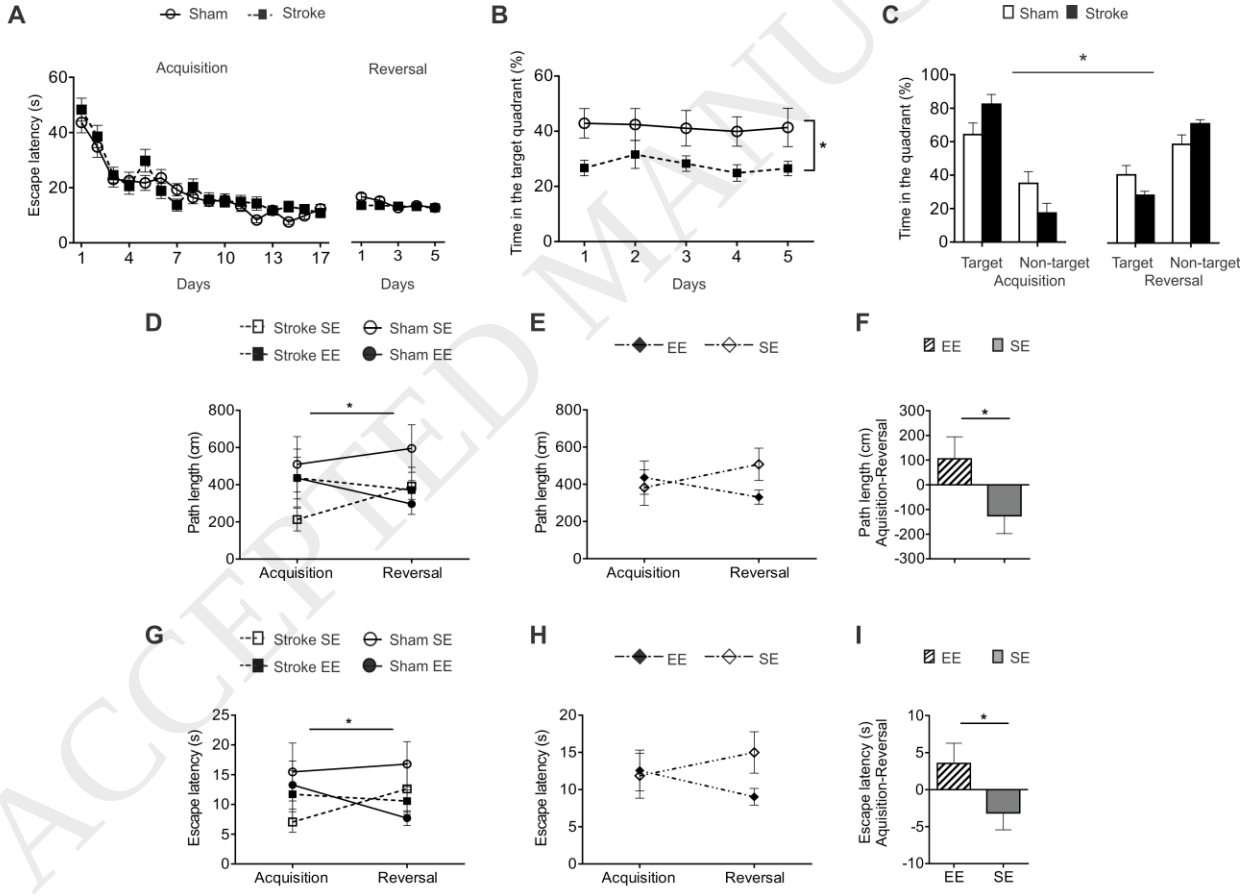

Figure 3: Post-stroke performance on egocentric navigation in the MWM test. (a) Performance on escape latency across days for acquisition and reversal learning (NS, $p > 0.05$). (b) There was a significant effect of surgery on percentage of time spent in target quadrant. Post hoc showed that stroke rats exhibited impaired behavioural flexibility during 5 days of reversal learning ($p < 0.05$). (c) Acquisition and reversal probes revealed a time effect ($p < 0.01$) on percentage of time

in the target quadrant. Animals spent more time in the target quadrant during acquisition and more time in the non-target quadrant in the reversal. (d,g) Path length and escape latency performance during acquisition and reversal periods for all experimental conditions detected a time and housing effect (*time*housing* effect $p < 0.05$). (e,h) No significant difference between enriched and non-enriched groups at either acquisition or reversal were observed when these time points were considered separately. (f,i) On the other hand, the mean difference between acquisition and reversal revealed that enriched animals had better performance in both path length and escape latency compared to non-enriched animals ($p < 0.05$). Data is expressed as mean ± SEM. For panels a,b,c Stroke n=12, Sham n=15. For panels d and g Stroke SE n=6 (3 males and 3 females), Stroke EE n=6 (4 males and 2 females), Sham SE n=8 (4 males and 4 females), Sham EE n=7 (3 males and 4 females). For panels e,f,h,i SE n= 14 (7 males and 7 females) and EE n= 13 (7 males and 6 females).

4. Discussion

In this study, we investigated the potential of EE to restore cognitive function after mPFC stroke. We used an egocentric spatial version of MWM to evaluate behavioral flexibility and memory. The classic triad of EE factors - social interaction, physical and cognitive activities – was available to enriched rats for 2 hours per day for 5 weeks. The social interaction duration and number of entries to the running wheel and food reward cage were recorded using an RFID tracking system.

We observed that mPFC stroke animals spent a lower proportion of time on the top floor of the EE cage where the running wheel was available, and as such, they engaged in less running wheel activity compared to shams. Further, on week 5 of EE, mPFC stroke rats spent a greater proportion of their time on the bottom floor, which contained assorted toys that were changed weekly. While animals given mPFC stroke showed no spatial learning impairment, they had impairments when performing a new navigation strategy during the reversal learning of the MWM task, demonstrating a deficit in behavioral flexibility. Rats exposed to EE solved the maze faster using shorter swim distances after reversal compared to non-enriched animals. The increased ability to solve the task may be linked to physical activity engagement in EE, especially in the sham rats, that presented higher number of running wheel entries compared to stroke. As mentioned above, stroke rats engaged less in running wheel activity than shams while in EE. Lack of motivation and fatigue are frequently reported in clinical studies as exercise barriers for stroke survivors[48,49]. Reduced activity levels and fatigue have also been reported early on after photothrombotic stroke in mice[39]. The running wheel was not the only modality of physical activity available in our EE housing. Ladders, beams, and unstable ramps were also

available as forms of exercise, but specific engagement in these activities was not monitored and quantified by our RFID tracking system.

Overall, the middle floor was the most visited area in the EE cage. On this floor we included a novel digging apparatus that provided a palatable food reward in different digging media. Although the RFID system detected which rat was inside the box that contained the digging pots, it was not possible to infer attention and cognitive flexibility with our present design. Additionally, the RFID tracking system used here tracked the locations of all rats throughout each EE session, and was used to determine the amount of time rats were in the same location together. The social environment of EE allows the expression of innate rodent social behaviors such as fighting, following/chasing and rough-and-tumble play[44,50]. We showed that rats spent most of the enrichment time at the same location, likely indicating an increased probability of social interaction. The present study did not show a relationship between social interaction and cognitive improvement, although it could be argued that less time alone could increase other cognitive and physical activities. Others have suggested that providing a social environment has an important role in promoting recovery after an ischemic brain injury, especially when housing sham-operated and stroke animals together[51]. However, this sort of analysis has been performed with enrichment and motor recovery after stroke. In these studies, it is evident that no single component is key for improved motor recovery, instead, it is the combination of elements (e.g. socialization, exercise, sensory-motor stimulation, task specific training) that synergize to promote recovery of motor function[28,34,42,52]. However, despite the evidence from EE animal studies, where animals experience a high levels of social interaction, cognitive stimulation and opportunities for physical activity, in the present clinical setting stroke patients spend most of their time inactive and alone[34,49]. Emulation of preclinical EE in patient care may ameliorate the physical inactivity and relative isolation of stroke patients that is often described in acute stroke units[34,43,53].

We demonstrated that a low-cost RFID tracking system (~500 CAD) can be integrated into an enriched housing environment and improve the assessment of spontaneous “free-activity” and its contribution to cognitive function following stroke. Information about individualized rat location over time can be used to determine activity preferences (e.g. wheel running, food reward experiences, task-specific training) and social interaction without experimenter influence and could be a powerful tool for identifying additional predictors of post-stroke impairment and

recovery. Integration of video recording with the RFID tracking data could increase analysis potential, particularly to distinguish between social behaviours (e.g. following/chasing and rough-and-tumble play)[45]. Nevertheless, our study highlights that both physical activity and social interaction play a role in post-stroke recovery. Our findings suggest that physical activity is a more important component of EE treatment than social interaction on cognitive flexibility. Stroke-induced physical inactivity, reflected by reduced access to the running wheel and time spent on the top floor, is a new finding in this mPFC stroke model. The reduction in physical activity is not due to a generalized motor impairment since we have previously shown that similar strokes do not impair spontaneous limb use or skilled reaching for food pellets[54].

It is important to note that we detected two error sources in the RFID tracking system, false readings due to rats lingering around the RFID sensor, and secondly, the spatiotemporal resolution of the RFID technology we employed was too low to detect high-speed movements[44,45]. A solution to improve tracking accuracy would be the use of motorized gates, based on RFID-presence detectors, to block the passage and restrict the rat towards a compartment or activity[55]. Future studies should also integrate a low-cost automated feeder[40,56] (e.g. Arduino microcontroller) to provide controlled food reward to stimulate self-training on a cognitive task in the home cage. The growing use of touch-screen platforms to accurately evaluate attention and cognitive flexibility could potentially also be adapted to the EE cage[57,58].

In conclusion, this study explores the potential of individualized tracking of rat activity using RFID-tracking to document different behaviors during EE exposure in animals recovering from mPFC stroke. We demonstrate that mPFC stroke impairs reversal learning of egocentric MWM and also reduces engagement in running wheel activity in EE. Notably, the use of EE as a rehabilitation intervention improved cognitive flexibility in both stroke and sham rats. The data obtained in the current study, represents the first steps in developing a low-cost automated rodent post-stroke assessment device to address questions such as the role of social interaction, physical and cognitive activity on cognitive rehabilitation. Moreover, this study represents an attempt to better align preclinical and clinical implementations of EE and facilitate the uptake of this intervention in the clinical setting.

Funding

This work was supported by CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil and the Canadian Partnership for Stroke Recovery (CPSR).

Acknowledgments—The authors would like to thank Dr. Gergely Silasi and Dr. Eduardo Balbinot for technical assistance. We also thank Anthony Carter for animal surgeries and MRI imaging acquisition.

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