



Research report

Assessing cognitive function following medial prefrontal stroke in the rat



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HIGHLIGHTS

- Bilateral intracerebral injections of endothelin-1 produce consistent damage to the mPFC.
- mPFC damage results in behavioural deficits in a number of cognitive functions.
- Functional deficits persist in the chronic post-stroke phase, representing a useful time frame for evaluating potential interventions.

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ABSTRACT

Cognitive impairments are prevalent following clinical stroke; however, preclinical research has focused almost exclusively on motor deficits. In order to conduct systematic evaluations into the nature of post-stroke cognitive dysfunction and recovery, it is crucial to develop focal stroke models that predominantly affect cognition while leaving motor function intact. Herein, we evaluated a range of cognitive functions 1–4 months following focal medial prefrontal cortex (mPFC) stroke using a battery of tests. Male Sprague–Dawley rats underwent focal ischemia induced in the mPFC using bilateral intracerebral injections of endothelin-1, or sham surgery. Cognitive function was assessed using an open field, several object recognition tests, attentional set-shifting, light–dark box, spontaneous alternation, Barnes maze, and win-shift/win-stay tests. Prefrontal cortex damage resulted in significant changes in object recognition function, behavioural flexibility, and anxiety-like behaviour, while spontaneous alternation and **locomotor** function remained intact. These deficits are similar to the cognitive deficits following stroke in humans. Our results suggest that this model may be useful for identifying and developing potential therapies for improving post-stroke cognitive dysfunction.

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1. Introduction

Anterior cerebral artery (ACA) stroke accounts for approximately 3% of strokes [1–3]. The resulting damage to the frontal lobes produces deficits in learning, memory, and executive functions including planning and cognitive flexibility [4–6]. Such impair-

ments can persist for years [7] and are associated with higher rates of long-term post-stroke disability [8,9] and increased burden on caregivers [10,11].

Traditionally, preclinical stroke research has focused almost exclusively on motor impairments chiefly because these deficits are more obvious and easier to study in animal models. The most widely used model of focal ischaemia in rodents, the middle cerebral artery occlusion (MCAo) model [12], predominantly affects sensory-motor circuits, a major confounding factor when testing cognitive functions in animals. However, with the development of focal stroke models such as the endothelin-1 (ET-1) model, it is

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possible to target specific regions of the brain involved in cognition while leaving motor function intact [13]. Using such a model, it is possible to conduct systematic evaluations into the nature of post-stroke cognitive deficits and ensuing recovery, and to determine how these non-motor brain areas respond to post-stroke treatments such as rehabilitation or drug therapy.

The medial prefrontal cortex (mPFC) has been implicated in a variety of cognitive and executive processes, including working memory, decision-making, inhibitory response control, attentional set-shifting, and temporal integration of behaviour [14]. As in humans, the rat mPFC consists of anatomically distinct sub-regions including the prelimbic, infralimbic, and anterior cingulate cortices [15,16]; however, the extent to which discrete cognitive processes can be attributed to these regions in rodents remains controversial [17,18]. Various non-ischaemic lesion models have established the importance of the mPFC in executive functioning, including complex sequences of behaviour that involve planning, problem-solving, and task flexibility [19–25]. Recently, Endepols et al. [26] characterized an ischemic model of anterior cerebral artery occlusion that largely affected prefrontal regions of the brain, and resulted in context-dependent changes in executive function on food foraging behaviour. Similarly, we have previously shown deficits in extradimensional set-shifting using a paradigm incorporating several intra- and extra-dimension set shift challenges in an ET-1 model of mPFC ischaemia [13]. Here, we were interested in characterizing the behavioural impairments in this stroke model more fully, especially during the chronic post-stroke phase.

To this end, we evaluated a range of cognitive functions 1–4 months after focal mPFC stroke in the rat. Herein we describe performance in the open-field, several object recognition tests, attentional set shifting, light/dark box, spontaneous alternation, a modified Barnes maze paradigm, and win-shift/win-stay tests.

2. Methods and materials

2.1. Subjects and experimental timeline

Adult male Sprague–Dawley rats ($n=25$) were purchased from Charles River Laboratories (Montreal, Canada) and pair-housed on a 12 h reverse light/dark cycle (lights off at 08:00). All procedures took place during the dark cycle, as activity levels have been shown to affect performance on tests [27]. Upon arrival to the facility, animals were allowed to acclimate for four days, and then were handled daily to familiarize them with experimenters. Animals had ad libitum access to food and water except during pre-stroke training and during the appetitively-motivated attentional set shifting and win-shift/win-stay testing periods, described below. Animals weighed between 250 and 300 g at the time of surgery. Following surgery, animals were handled regularly over the following 3 weeks. Testing began on post-surgical day 24. A detailed timeline of the experiment is presented in Fig. 1. All procedures were conducted in accordance with the guidelines of the Canadian Council for Animal Care, and were approved in advance by the University of Ottawa Animal Care Committee.

2.2. Surgical procedure

Endothelin-1, a vasoconstrictive peptide, was used to produce focal ischaemia as described previously [13]. Anaesthesia was induced using 4.0% isoflurane, and rats were maintained with 2.0% isoflurane during surgery. Once anesthetized, animals were mounted in a stereotaxic apparatus (David Kopf Instruments, USA), and the scalp was incised and retracted. The surface of the skull was cleared, and small drill holes were made in the skull above the mPFC, at bilateral coordinates from bregma [AP + 3.5, ML \pm 0.6, DV

–5.2] and [AP + 2.5, ML \pm 0.6, DV –5.0] (DV measured from brain surface) [16]. A Hamilton syringe (26 gauge; Hamilton, Nevada, USA) was carefully lowered at each location and left undisturbed for 1 min. Endothelin-1 (each injection = 0.8 μ l, 400 pmol/ μ l in sterile water; Calbiochem, California, USA) was injected at a flow rate of 0.4 μ l/min, and the needle was left undisturbed for 2 min before being slowly retracted from the brain. The scalp was sutured and the incision site was treated with topical anaesthetic (2% Bupivacaine, 0.1 ml, Chiron, Ontario, Canada). Body temperature was monitored continuously and maintained at 37.0 ± 0.2 °C for the duration of surgery using a heating pad. Following surgery, animals were placed into a 37 °C incubator until awake and active (approximately 30 min), then given a subcutaneous injection of buprenorphine (0.05 mg/kg; Chiron) and returned to their home cages. Sham animals were treated identically to stroke animals, except no craniotomy was performed.

2.3. Behavioural testing

Following daily handling, animals were pre-trained on the attentional set shifting task (ASST) described below. All other testing took place post-surgery. On the day of each behavioural test, animals were allowed to acclimate to the behavioural testing suite for at least 30 min. Because testing took place during the dark cycle, animals were kept in the dark at all times except during testing, which took place under normal room lighting unless otherwise stated. All tasks requiring video analysis were analyzed using Ethovision XT[®] software (v. 10; Noldus Information Technology, Netherlands). All behavioural testing was performed by an experimenter blind to surgical condition.

2.3.1. Attentional set shifting task

For the duration of training, and later testing, rats were food restricted to 90–95% body mass. The ASST was performed as described by others [24,28,29] using a T-maze containing inserts of varying colour (black and white) and texture (rough and smooth). The apparatus (Med Associates, USA) was a configuration of 4 transparent arms (45 \times 10 cm) covered with white cardboard to minimize visual distraction, with a central platform (11.5 \times 11.5 cm). Each arm contained an insert with a unique colour/texture combination, wherein two arms had white floors and two arms had black floors; one of each was smooth textured, and the others rough. Training took place over 7 days. On the first day, animals were placed into the maze with all arms open and several palatable sugar pellets (45 mg; TestDiet, Missouri, USA) scattered throughout the maze. After free exploration for 10 min, animals were returned to their home cages. On subsequent training days, the number of pellets scattered throughout the maze and the time of exposure were gradually reduced, until on the fourth day pellets were located only in the food cups at the ends of each arm during 5 min of exploration. Once animals were familiar with the location of the reward cups, the remaining training days consisted of an equal number of randomly assigned starts from each of the four arms, with the opposite arm blocked and both possible choices rewarded. Following 7 days of training, most animals were able to enter the maze and make a choice to either arm within 1 min. Some animals ($n=3$) required three additional training sessions to reach this level of performance. During training, all arms choices were always baited so no associations could be made between colour/texture and reward.

Three weeks following surgery, animals underwent one re-acclimation session to the maze, identical to the last training day. The following week, they began the following 2-day extradimensional set-shifting paradigm. On the first day, animals were randomly assigned to learn to associate either black arms or white arms with reward, regardless of texture. Animals were given up to

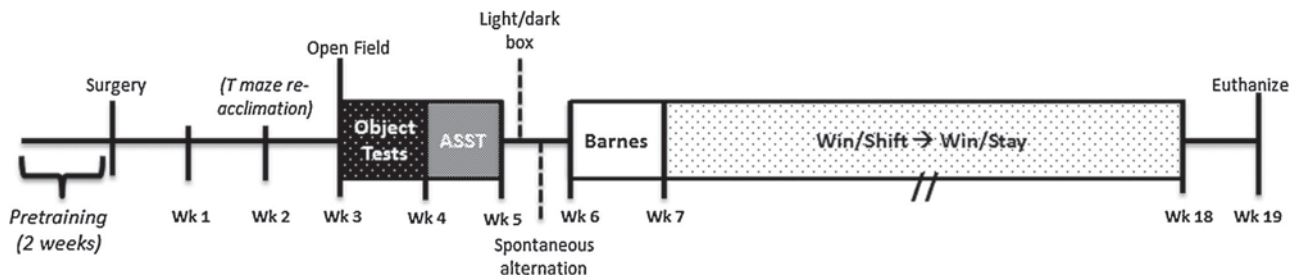


Fig. 1. Experimental timeline.

Following arrival at the facility, animals were pre-trained on the Attentional Set Shifting Task (ASST). Following surgery, animals were handled regularly for three weeks, then underwent open-field testing, followed by temporal object, object/context, and object/placement recognition tests, then ASST. The following week they were tested once in the light/dark box, and two days later on spontaneous alternation. The following week, Barnes maze acclimation and testing took place. Following that, win-shift/win-stay testing began, and continued until criterion (or maximum time allowed) was reached. Animals were euthanized at 19 weeks post-surgery.

120 trials to reach a criterion of 8 consecutive correct choices. The trials were performed in blocks of 8, during which rats would start from each arm an equal number of times. Each trial consisted of entering the start arm (with the opposite arm blocked), traversing the maze, and entering one of the two choice arms to consume the sugar pellet reward. The maze was rotated every 2 blocks to minimize the use of spatial cues in the room. Between trials, animals were placed into a holding cage for 15 s while the maze was prepared for the next trial. The number of trials to criterion and the number of errors made were recorded. Once criterion was reached, animals were removed to their home cage.

The following day, animals were randomly assigned to associate either smooth or textured arms with reward, regardless of colour. The assignment of texture was made irrespective of the colour assigned on the previous day. Testing was performed as described above. The number of trials to criterion and the number of errors made were recorded for Sets 1 and 2. During Set 2, the proportion of errors made wherein rats chose to enter an arm that would have been correct according to Set 1 criteria were recorded (perseveration errors). Once criterion was reached, animals were returned to ad libitum feeding.

2.3.2. Open field

Animals were placed into the centre of a novel square arena (100 × 100 cm) and allowed to explore for 10 min. The first five minutes were recorded and analyzed to determine distance moved in the perimeter and middle of the maze, the number of times animals crossed from the perimeter to the middle zones, and the amount of time spent in the corners of the maze. The zones were defined by dividing the maze into a 4 × 4 grid using Ethovision® software, with each square measuring 25 × 25 cm. Thus, the centre was the innermost 4 squares and the perimeter consisted of the squares on the edge of the field. The open field sessions also served as an acclimation exposure for later object recognition testing in the same apparatus (described below).

2.3.3. Temporal object recognition

Following 2 × 10 min daily exposures to the apparatus used for open field testing, animals were tested for temporal object recognition (TOR). This task comprised two exposure phases followed by a test phase, each separated by 1 h, similar to a paradigm described previously [30]. In the first exposure phase, animals were placed into the apparatus and allowed to explore two identical objects for a total of 4 min. A different pair of identical objects was used in the second exposure phase. During the test phase, one copy of each object was placed into the apparatus and animals were given 3 min to explore (Fig. 2A). The order of objects used in each exposure phase was randomized. The amount of time spent exploring the objects in the exposure phases and in the test phase was determined by video analysis. Object exploration was defined as the

animal directing its nose towards the object within 5 cm. If the animal rested on the object, this was not considered active object exploration.

2.3.4. Object context recognition

This task was modified from that described previously in mice [22]. The test comprised 2 distinct contexts, using different apparatuses in different rooms, with varied lighting in each. Testing was performed following at least 2 × 10 min daily exposures to each context. Context A consisted of the apparatus used for the open field test, which had a grey floor and walls and was housed in a well lit room (280 lx). Context B consisted of a 100 cm diameter round maze with white walls and a black floor, set up in a dimly lit room (30 lx). Each context was associated with a unique pair of identical objects (all objects were different from those used for TOR testing). Animals were given two consecutive exposure sessions (4 min), one in each context, followed 5 min later by a test session (3 min). The test session took place in one of the contexts and contained one copy of the object associated with Context A and one copy of the object associated with Context B (Fig. 2B). The order of context exposure and the context used for the test session were randomized. The amount of time spent exploring the objects in the exposure phases and in the test phase was defined as above and determined by video analysis.

2.3.5. Object placement recognition

The object-in-place test was performed as described previously [23], in the apparatus used for open field testing. Animals were given one exposure phase followed immediately by a test phase. In the exposure phase, animals were allowed to explore four different objects (A–D; all different from objects used in TOR and context recognition) for 5 min. Then, the positions of two of the objects were exchanged (either A and B, or C and D), and the animals were placed back into the maze for a 3 min test phase (Fig. 2C). The pair of objects that were switched was randomized. The amount of time exploring each pair of objects in the exposure phase and in the test phase was defined as above and determined by video analysis.

2.3.6. Light/dark box

Animals were placed into a light/dark box (LDB) apparatus that consisted of a clear exposed portion (61 × 46 cm) and a black enclosed portion (30 × 46 cm) accessed via an entry hole. Without prior exposure, animals were placed into the apparatus for 5 min, and the amount of time spent in the exposed light area and the dark enclosed area were determined by video analysis.

2.3.7. Spontaneous alternation for turning bias

This task was performed using a T-maze apparatus constructed from corrugated plastic. The maze consisted of a long start arm (20 cm × 100 cm), at the top of which were perpendicular choice

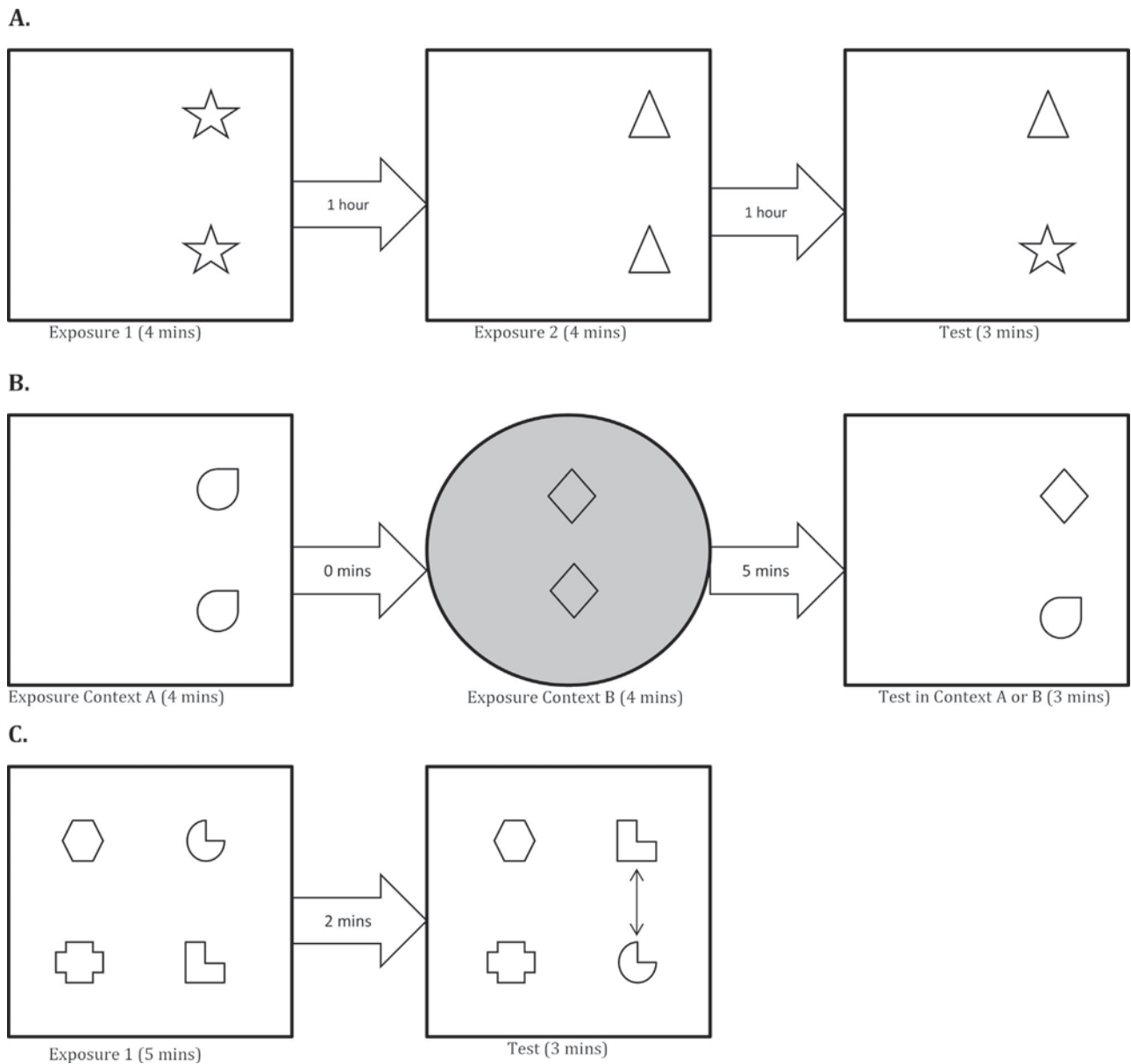


Fig. 2. Object recognition tests.

Temporal object recognition was tested by exposing animals to two different pairs of identical objects 1 h apart, then testing the preference for one of each object 1 h later (A). Context memory was tested by exposing animals to distinct pairs of objects associated with unique contexts, then testing the preference for each object when one appeared out of context (B). Object placement memory was tested by exposing animals to four unique objects, then swapping the position of two and determining preference for either the in-place or out-of-place pair (C). * $p < 0.05$.

arms (15×40 cm). At the top of the start arm, a central dividing partition extended 15 cm into the stem, forcing the right or left arm choice prior to reaching the convergence point [31]. Animals were placed into the maze at the bottom of the start arm, and allowed to traverse the maze and choose a direction. Choice was recorded once all four paws had entered the arm. Animals were given 7 trials separated by 30 s, for a possible total of 6 spontaneous alternations between right and left choices.

2.3.8. Barnes maze

Two days prior to testing, animals were first exposed to three training trials during which they were required to find a goal box located at a randomly chosen escape hole. Each animal was placed into the centre of the maze, and aversive light and noise stimuli were applied. The animal was given up to 120 s to find and enter a randomly positioned goal box, at which point the aversive stim-

uli were terminated. All animals reliably entered the escape hole within the 120 s limit after completing the three acclimation trials.

Subsequently, testing took place over three days, each consisting of 5 consecutive trials with a 60 s inter-trial interval. Notably, the goal box was moved to a new randomly-assigned location each test day, in order to assess performance over the course of 5 trials on each of the three days. The number of approaches to incorrect holes (errors), the number of re-entries to previously visited holes, and the deviation from the correct hole on the second to fifth trials were recorded.

2.3.9. Win-shift/win-stay task

For the duration of testing, rats were food restricted to 90–95% body mass. The win-shift/win-stay task was performed using the same apparatus as the ASST. The wall coverings were removed, and standard commercial blue plastic arm inserts were used in place of the coloured textured inserts used for ASST. Because of the exten-

sive training and acclimation that had taken place as part of the ASST protocol, no further training was needed for this task.

Each day consisted of 10 paired trials: a forced choice, followed by a free choice. For the win-shift portion of the task, rats were placed in the start arm of the maze while one choice arm was blocked, forcing them down a randomly assigned arm baited with a sugar pellet. Immediately upon retrieving the pellet, animals were removed and placed into a holding cage for 15 s, while the blocked arm was opened and baited, and then placed back into the maze with free access to both arms. A trial was considered successful when rats chose the arm not previously visited. Criterion for the win-shift portion of the test was considered performance of $\geq 85\%$ over 4 consecutive days, up to a maximum of 25 days.

Upon completion of the win-shift task, rats were given one rest day, and then switched to win-stay testing. Here, the rats were required to choose the arm that was previously baited on the forced-choice trial. Due to the increased difficulty of this task (countering the instinctive response to search the arm not previously visited), criterion was considered a performance of $\geq 80\%$ over 3 consecutive days, up to a maximum of 50 days. Once individual animals had reached criterion, they were removed from testing but continued food restriction and handling equivalent to the animals that continued testing.

2.4. Histology

Following the completion of behavioural testing, animals were anesthetized with Euthanyl (i.p. 150 mg/kg; Bimeda-MTC Animal Health Inc., Ontario, Canada) and transcardially perfused with heparinized saline followed by 4% paraformaldehyde (PFA). Brains were extracted and post-fixed in 4% PFA overnight. They were then transferred to 30% sucrose in PBS (w/v) until saturated, frozen, and stored at -80°C . Brains were sectioned ($40\ \mu\text{m}$) using a cryostat and stained with cresyl violet to visualize infarcts. A minimum of 8 evenly spaced sections from each animal were used to estimate infarct volume using Stereoinvestigator[®] (v.11; MBF Bioscience, VT, USA). Ischaemic injury was defined as pallor, abnormal tissue architecture, and apparent necrosis indicated by a lack of cresyl violet staining [32]. The total volume of injury was estimated by summing the area of damage recorded from each section and multiplying that value by the distance between measured sections [13].

2.5. Statistical analyses

Statistical analyses were performed using SPSS (v.22; IBM Corporation, USA). Open field, ASST, LDB, and spontaneous alternation performance were analyzed using independent sample *T*-tests. Win-shift/win-stay performance was analyzed by Chi-square (overall number of animals reaching criterion), independent sample *T*-tests (trials to criterion), and Kaplan–Meier with Breslow generalized Wilcoxon comparisons (proportion of animals reaching criterion over time). Object recognition tests were analyzed by comparing exploration of test objects using Student's paired *T*-tests for each group. Barnes maze was analyzed using repeated measures ANOVA followed by independent *T*-tests when warranted. When data violated sphericity assumptions (Mauchly's test of sphericity), the Greenhouse–Geisser correction was applied (degrees of freedom rounded to the nearest integer). Statistical significance was considered $p < 0.05$. Values are expressed as mean \pm standard error of the mean (SEM).

3. Results

3.1. Histology

Two animals from the Stroke group died during surgery. The remaining animals constituted $n = 12$ Sham and $n = 11$ Stroke. In

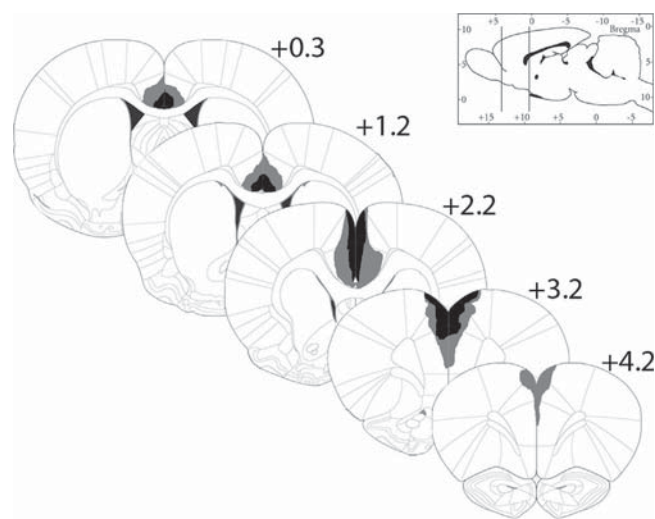


Fig. 3. Histology.

Representative injury profiles in mPFC damaged animals. Black areas represent typical minimum damage, while grey areas represent maximum damage at various stereotaxic coordinates.

Adapted from [16].

Stroke animals, there was bilateral damage typically affecting the prelimbic and cingulate cortices, between 4.22 and 1.34 mm anterior to bregma, with an average volume of $8.52 \pm 1.39\ \text{mm}^3$. In two subjects, the damage extended caudally to the medial cingulate cortex at -0.46 mm from bregma. The corpus callosum was intact in all but one subject, who sustained minor damage. There was occasionally limited damage to the medial caudal secondary motor area, and one animal exhibited unilateral ventricular hypertrophy. Representative maximal and minimal damage at various stereotaxic levels is presented in Fig. 3.

3.2. Behavioural testing

3.2.1. Open field

Animals in the Stroke group spent significantly less time in the corners of the maze compared to Sham animals (194 ± 4.1 vs. 210 ± 6.1 s, respectively; $t_{21} = 2.091$, $p = 0.049$) (Fig. 4A). There was no difference in the number of crossings from the outer edge of the maze to the centre area between groups (Sham 1.7 ± 0.70 vs. Stroke 4.8 ± 1.5 crossings; $t_{14} = -1.936$, $p = 0.073$) (Fig. 4B) or the total distance travelled by Sham and Stroke animals during open field testing (Sham 36.4 ± 2.9 m vs. Stroke 42.1 ± 6.8 m; $t_{21} = -1.563$, $p = 0.133$) (Fig. 4C).

3.2.2. Object placement tests

In all object tests, Sham and Stroke animals spent an equivalent amount of total exploration time during the test phases ($p = 0.118$), and did not exhibit a bias towards any particular object during the exposure phases ($p = 0.081$).

3.2.3. Temporal object recognition

Paired *T*-tests revealed that Sham animals spent an equal proportion of their exploration time examining both the first and second exposure objects ($t_{11} = -1.231$; $p = 0.122$), indicating that the 1 h time interval used in testing was not sufficiently long enough to induce a novelty response to the initially viewed object. However, stroke animals spent significantly more time exploring the less recent object ($t_{10} = -2.768$; $p = 0.010$) (Fig. 5A).

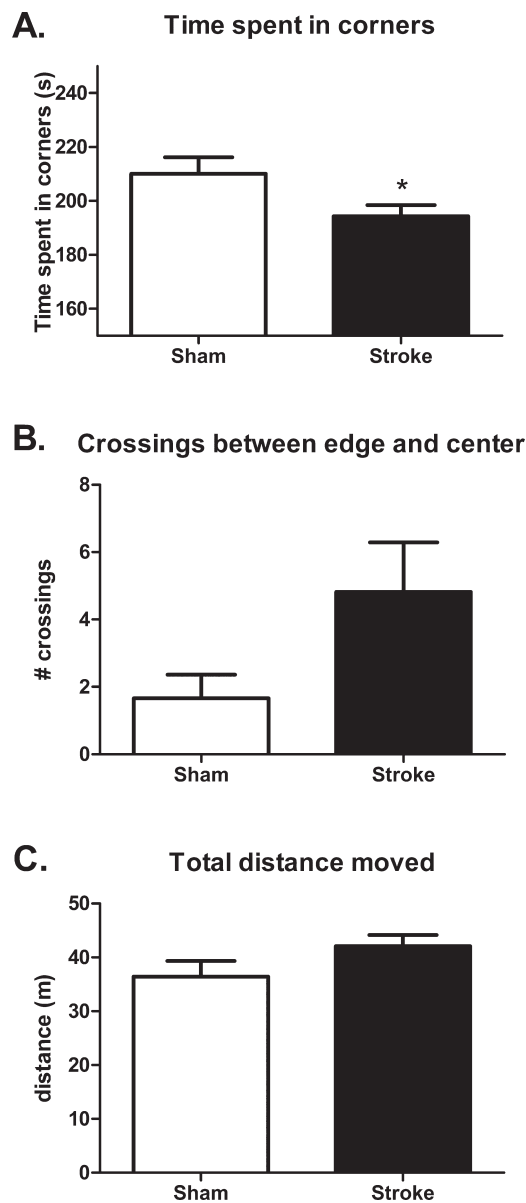


Fig. 4. Open field.

Analysis of performance in the open field revealed that Stroke animals spent less time in the corners of the maze compared to Sham animals (A). There were no statistical differences between groups in the number of times animals crossed between the perimeter and centre of the maze (B) or the total distance moved during the 5 min session (C). * $p < 0.05$.

3.2.4. Object context recognition

During the test phase, Sham animals spent significantly more exploration time investigating the object that appeared in a different context from the original exposure ($t_{11} = -2.551$; $p = 0.013$). Stroke animals, however, spent an equal proportion of time exploring both the in-context and out-of-context object ($t_{10} = -1.329$; $p = 0.107$) (Fig. 5B).

3.2.5. Object placement recognition

Following the exchange of two objects from their original positions, Sham animals spent significantly more exploration time on the objects that had been moved compared to the objects that were in their original location ($t_{11} = -1.873$; $p = 0.044$). Stroke animals spent equal proportion of time exploring both pairs of objects ($t_{10} = 0.559$; $p = 0.294$) (Fig. 5C).

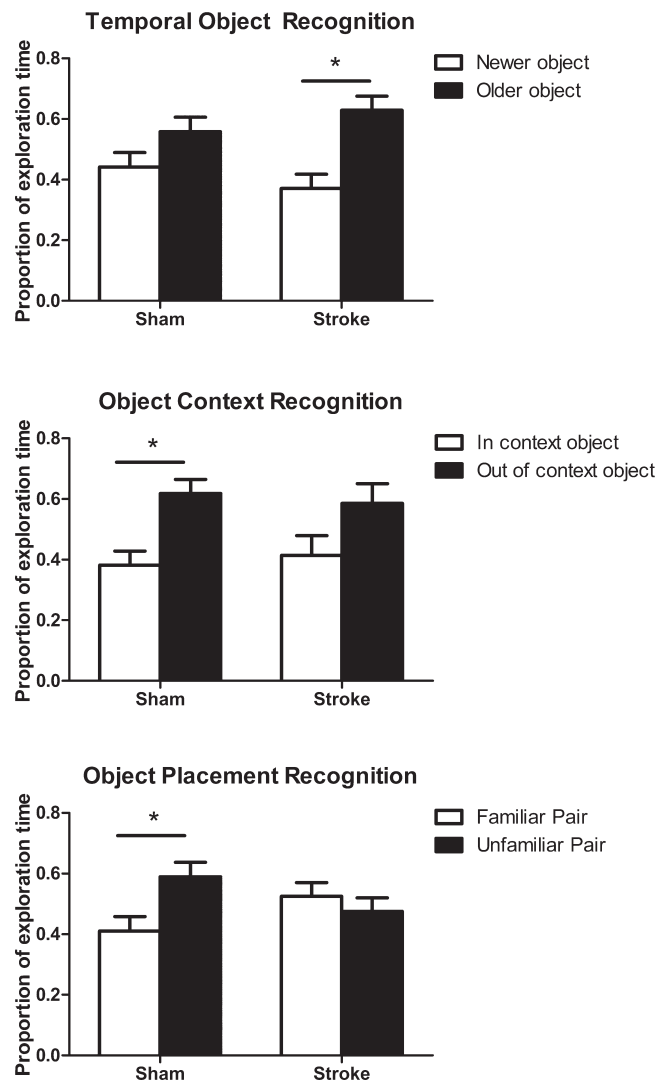


Fig. 5. Object recognition tests.

Sham animal did not make a distinction between the older and more recent object, suggesting both objects were considered relatively familiar. Sham animals, however, spent longer exploring the object presented first, suggesting a difference in temporal memory processing compared to Shams (A). In the object context (B) and object placement (C) tasks, Sham animals recognized the out-of-context or out-of-place objects, while Stroke animals did not spend more of their exploration time on those objects. * $p < 0.05$.

3.2.6. Attentional set shifting task

There was no difference between the number of trials to criterion for the first set (67.2 ± 9.47 trials for Sham vs. 45.6 ± 5.63 trials for Stroke; $t_{21} = 1.91$; $p = 0.07$), or the second set (53.0 ± 7.74 trials for Sham vs. 47.4 ± 6.23 trials for Stroke; $t_{21} = 0.560$; $p = 0.581$). Similarly, the number of errors being made before criterion was reached did not differ between groups [Set 1: Sham = 22.8 ± 3.63 vs. Stroke = 15.7 ± 2.34 errors ($t_{21} = 1.611$, $p = 0.122$); Set 2: Sham = 17.3 ± 3.05 vs. Stroke = 15.7 ± 2.34 errors ($t_{21} = 0.391$, $p = 0.700$)]. Lastly, there was no difference in the proportion of perseveration errors when shifting from set 1 to 2 (Sham = 66.4 ± 4.78 vs. Stroke = $59.1 \pm 4.60\%$ perseveration errors; $t_{21} = 1.093$, $p = 0.287$). A summary of ASST performance is presented in Table 1.

3.2.7. Light/dark box

There was no difference between groups in the amount of time spent in the light (13 ± 2 for Sham vs. 17 ± 3 s for Stroke, respec-

Table 1
Attentional set shifting test. There were no differences between the number of trials to criterion, the number of errors perpetrated before reaching criterion, or the nature of the errors that were made when switching between sets between the Sham and Stroke animals.

	Set 1			Set 2		
	Sham	Stroke	p-Value	Sham	Stroke	p-Value
# Trials to criterion	67.2 ± 9.47	45.6 ± 5.63	$p=0.07$	53.0 ± 7.74	47.4 ± 6.23	$p=0.581$
# Errors	22.8 ± 3.63	15.7 ± 2.34	$p=0.122$	17.3 ± 3.05	15.7 ± 2.34	$p=0.700$
% Perseveration errors	–	–	–	66.4 ± 5.24	59.1 ± 4.60	$p=0.287$

tively; $t_{21} = -1.234$, $p=0.231$) and dark areas (47 ± 2 for Sham and 43 ± 3 s for Stroke; $t_{21} = 1.268$, $p=0.219$) of the apparatus.

3.2.8. Spontaneous alternation

The spontaneous alternation test revealed that there was no turning bias in either the Sham or Stroke groups (68.1 ± 0.04 and $66.5 \pm 0.06\%$ spontaneous alternations, respectively; $t_{21} = 0.221$; $p=0.828$) (data not shown).

3.2.9. Barnes maze

Overall errors, number of re-entries to previously visited holes, and the distance between the correct hole and that first approached (termed “deviation”) were collapsed over days, for comparison over trials. Repeated measures ANOVA revealed no differences in the overall performance over Trials 1–5 with respect to errors ($F_{4,236} = 0.574$; $p=0.659$; Fig. 6A) or re-entries ($F_{3,199} = 0.500$; $p=0.680$; Fig. 6B). There was no significant effect of time on overall deviation ($F_{3,196} = 0.972$; $p=0.407$), however, there was a significant group effect ($F_{1,67} = 5.605$; $p=0.021$), resulting from the

higher deviation scores in the Stroke group compared to Sham (Sham 3.4 ± 0.23 vs. Stroke 4.3 ± 0.24 holes from the goal box; $t_{274} = -2.528$, $p=0.012$; Fig. 6C and D).

3.2.10. Win-shift/win-stay task

One animal ($n=1$ Stroke) was removed from testing due to aggressive behaviour and reluctance to traverse the maze and enter a choice arm. In the remaining subjects, there was no difference in the number of days to criterion in the win-shift portion of the task (Sham = 9.6 ± 1.96 , Stroke = 10.2 ± 1.67 days to criteria; $t_{20} = -0.202$; $p=0.842$). In the more challenging win-stay portion of the task, $n=2$ Sham animals and $n=3$ Stroke animals did not reach criterion by 50 days [not significantly different; $X^2(1) = 0.552$; $p=0.457$]. Of the animals that did reach criterion, those in the Sham group took 391 ± 20.2 trials, while Stroke animals took significantly longer, 467 ± 13.8 trials ($t_{15} = -2.826$; $p=0.013$). Similarly, Kaplan Meier analysis revealed that there was no difference between the proportion of animals in each group reaching criterion over time in the win-shift portion of the task ($X^2_1 = 0.056$, $p=0.812$; Fig. 7A),

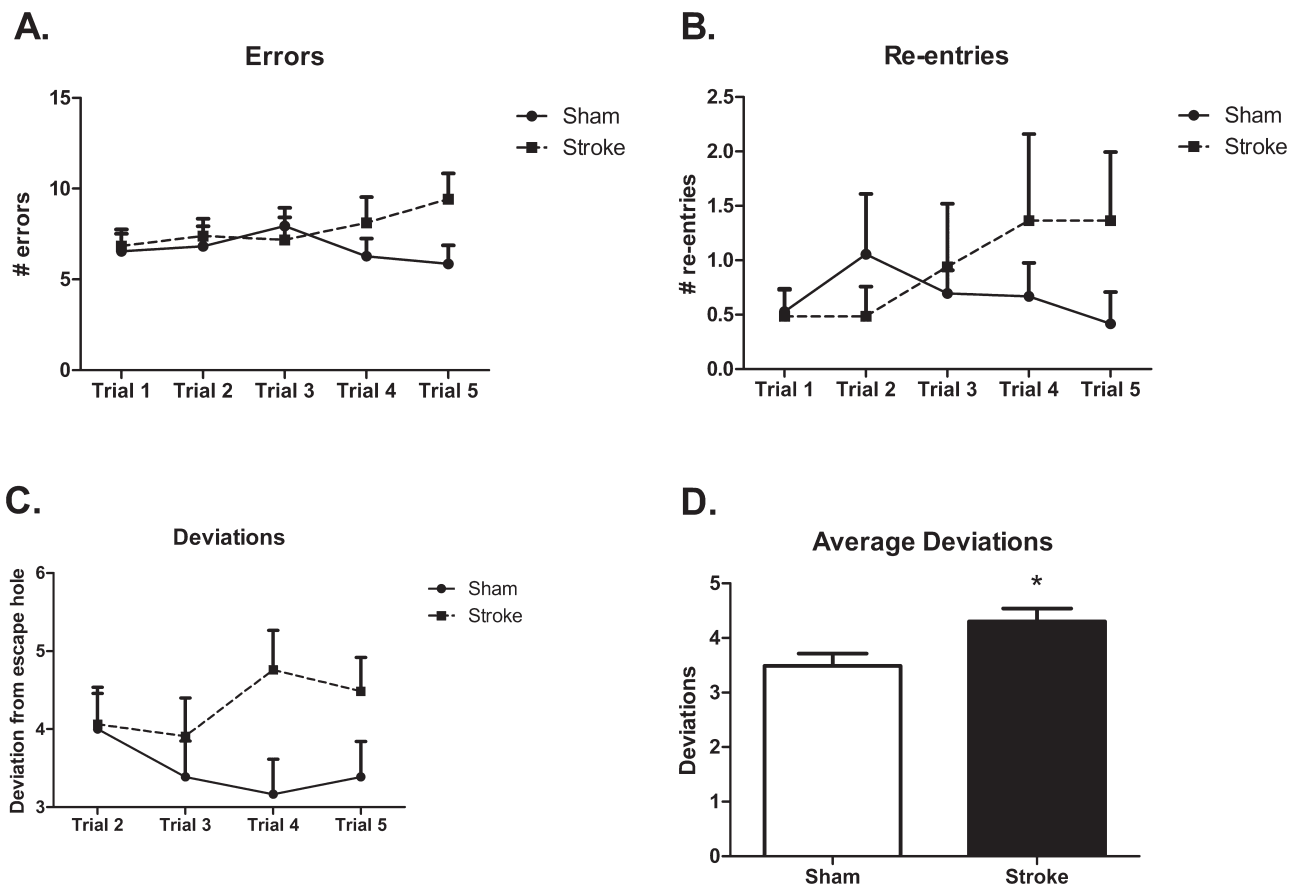


Fig. 6. Barnes maze performance.

When three Barnes maze testing days, each with discrete goal box location, were combined, analysis revealed that Sham and Stroke animals did not differ in the number of errors (A) or re-entries (B). There was no significant effect of time on overall deviation (C), however, there was a significant group effect wherein the Stroke group deviated significantly more than the Sham group from the daily goal location (D). * $p < 0.05$.

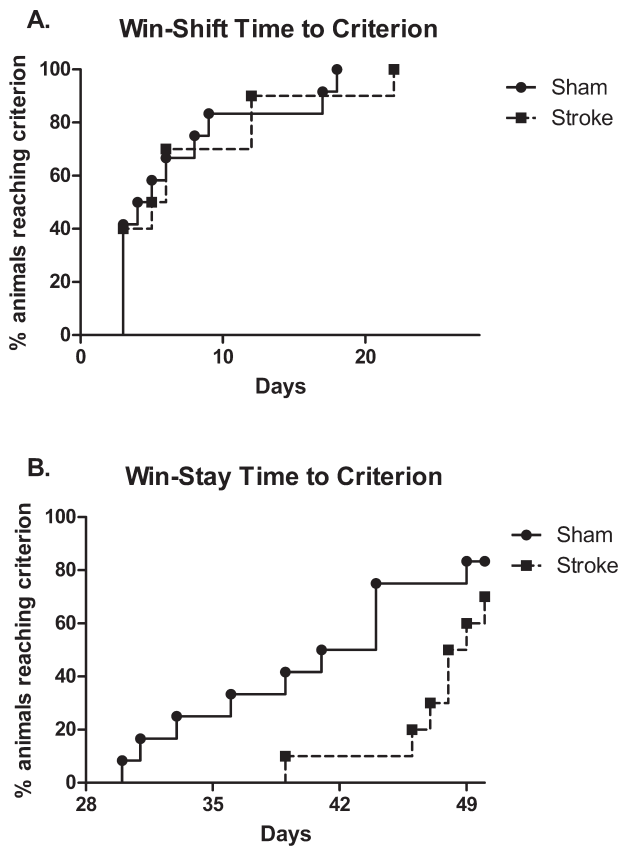


Fig. 7. Win-shift/Win-stay performance. There was no difference in the proportion of Sham and Stroke animals acquiring the win-shift portion of the task over time (A); however, Stroke animals took significantly more trials to reach criterion on the win-stay portion of the task (B).

while there was a significant difference between groups in the win-shift portion ($\chi^2_1 = 4.870, p = 0.027$; Fig. 7B).

4. Discussion

Due to the relative paucity of stroke models specifically assessing cognitive function, we performed this study to evaluate an mPFC stroke model using a battery of tests during the chronic post-stroke phase. Bilateral injections of ET-1 into the mPFC produced damage extending through the prelimbic and cingulate cortices (Fig. 3), that affected behaviour on a number of cognitive tests (Figs. 4–7), with no locomotor impairments (Fig. 4C) or bias in spontaneous alternation. Other similar studies from this lab have shown a similar lack of effect on motor function as assessed by skilled reaching and cylinder tests [33].

The lesions produced in this study measured on average $8.52 \pm 1.39 \text{ mm}^3$, which is smaller than our previous report using a similar model [13], perhaps due in part to the long post-stroke time frame used in this study and a somewhat more rapid infusion rate used in the present study. However, with the exception of damage to the infralimbic cortex, the brain regions affected in both studies were similar, and correspond to those reported in other non-ischemic mPFC models [19–21,23,25,34].

The deficits observed in this study were evident 1–4 months following injury, a time frame beyond that used in most mPFC injury lesion studies [19,21,22,26]. This time frame is particularly relevant for the evaluation of interventions to improve cognitive recovery, which may require treatment over a number of weeks or months.

The open field test was used to examine locomotion and anxiety-like behaviour. In general, Stroke animals traveled the

same distance as Shams (Fig. 4C); thus, motor impairments were not likely to confound the tests used in this study. Further, Stroke animals spent less time in the corners of the maze, consistent with a reduced anxiety phenotype. A similar result was reported following mPFC damage resulting from anterior cerebral artery occlusion [26].

We used several object recognition tests designed to probe temporal, spatial, and contextual memory processing. Interestingly, using a TOR protocol that consisted of 1 h intervals between object exposures and testing, the Sham group explored both the older and more recent object equally. In contrast, Stroke animals spent more time exploring the first exposure object. This suggests that in this short time interval, Sham animals regarded both objects as similarly familiar, while Stroke animals showed impaired recognition of the older object. This is in contrast to a similar paradigm used by Hannesson et al. [30], wherein a comparable time interval resulted in Sham animals exploring the less-recently presented object for longer, indicating recency discrimination. Perhaps this phenomenon would have been achieved herein by using a longer time interval (e.g. ≥ 3 h) between the exposures and test phase [23]. It would be interesting to examine how this time interval would affect performance of mPFC damaged animals, whose performance was significantly different from Sham animals on this task nonetheless.

Disruption in object recognition processing was observed in both the object-in-place and object context test. In both tests, Sham animals spent significantly more of their exploration time examining the objects that were switched/out-of-context, whereas Stroke animals spent equal time exploring all test objects. Similar deficits have been previously reported following aspiration of the prefrontal cortex in mice [22], and following excitotoxic lesions to the mPFC in rats [23].

We [13] and others [21] have previously described deficits in set shifting ability following mPFC damage using a multi-dimensional model of ASST. Here, we employed a simplified set shifting test using a T-maze. Animals were first taught to associate a certain dimension (colour) with food reward and, once learned, an extra-dimensional shift to a texture-based reward took place. Despite other reports supporting prefrontal mediation of set shifting ability using the T-maze colour/texture task [24,28,29], we did not observe any deficits in the performance of Stroke animals (Table 1). The discrepancy between these ASST results may be due to the lack of more complicated intra-dimensional, reversal, and multi-dimensional shifting paradigms, which allow for more set shifts and the incorporation of irrelevant ‘decoy’ dimensions. Nonetheless, the relatively laborious nature of the more complex ASST paradigm [21] (a single animal may require multiple full days of testing, depending on chosen set shifts) is an important consideration when performing ASST testing in experiments that involve multiple groups of animals (e.g. rehabilitation or drug intervention studies).

The Barnes maze paradigm used in the present study was unique, and designed to evaluate executive function by moving the goal box daily, then evaluating performance over the course of trials. This differs from more common Barnes maze paradigms designed to examine spatial learning over several days, primarily a hippocampal function [35]. We compared the total number of errors, the number of re-entries to previously visited holes, and how far from the goal box position the animals deviated during the second to fifth trials (following establishment of new daily location). While both Sham and Stroke animals made the same mean number of errors and re-entries to previously visited holes over trials, the Stroke animals exhibited a higher overall deviation score (Fig. 6A–D). This suggests a deficit in behavioural flexibility expected in animals with mPFC damage.

The win-shift/win-stay test revealed a significant deficit in the ability of animals to switch from the first to the second strategy, with no impact on acquisition of the first task. This further supports altered behavioural flexibility expected as a result of prefrontal damage [14,19,36]. However, it is also possible that the win-stay task is simply more difficult than the win-shift task and the deficits observed in this study may not reflect impaired behavioural flexibility. Previous studies from our lab employing the win-shift/win-stay test in other stroke models resulted in Sham animals learning both the win-shift and win-stay rules in significantly fewer trials compared to the present study [37,38]. This discrepancy could be due to interference from using the same apparatus for the ASST paradigm prior to the initiation of this test. In an attempt to diminish this possibility, the ASST and win-shift/win-stay tests were performed 2 weeks apart, the maze configuration was altered, and the apparatus was set up in a different testing room. Nonetheless, considering the difficulty that Sham animals exhibited in reaching criterion, only one of these tests should be chosen for future studies. Considering the significant deficit found in the win-stay test, despite the longer-than-expected time to criterion, this test represents a more sensitive measure of damage in this model compared to the T-maze ASST.

This study provides a comprehensive analysis of a number of cognitive abnormalities in the chronic phase following mPFC stroke. Object recognition functions, behavioural flexibility (or task difficulty), and anxiety-like behaviour were affected, while alternation behaviour and **locomotor** activity remained intact. This model reproduces some of the key characteristics of prefrontal stroke in humans, and thus may be helpful in designing new interventions to mitigate post-stroke cognitive impairment.

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