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Research report

A novel behavioural registration system LABORAS™ and the social interaction paradigm detect long-term functional deficits following middle cerebral artery occlusion in the rat

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Abstract

Following stroke, patients suffer a wide range of disabilities including motor impairment, anxiety and depression. However, to date, characterisation of rodent stroke models has concentrated mainly on the investigation of motor deficits. The aim of the present studies was therefore to investigate home cage behaviour (as assessed by a recently developed automatic behavioural classification system, LABORAS) and social behaviour (as a measure of anxiety) in rats following transient middle cerebral artery occlusion (tMCAO). Rats subjected to tMCAO (90 min) showed deficits in general home cage behaviours including locomotion, rearing, grooming and drinking for up to 7 weeks post occlusion, as compared with sham operated controls. In addition, a significant decrease in the total duration of social interaction was also observed in occluded rats compared with shams. The data shows that in addition to motor deficits, animals display changes in home cage behaviour and decreased social behaviour which, in contrast to motor function, are prolonged over time. Transient MCAO in rats may therefore provide a pre-clinical model to investigate agents offering symptomatic relief for ischaemia-induced motor deficits and anxiety over time following injury.

Theme: Disorders of the nervous system
Topic: Ischaemia

Keywords: Rat; MCAO; Neurological score; Automatic behaviour registration; Social interaction, anxiety

1. Introduction

Despite a large investment by academic and industrial laboratories, the only registered agent for the treatment of acute stroke is tissue plasminogen activator (tPA). Far from ideal, tPA is only effective when administered within 3 h after the onset of stroke and following a CT scan to eliminate haemorrhagic stroke where it is contraindicated [3,24]. To date, the majority of pre-clinical efforts have been directed towards developing agents aimed at ameliorating ischaemia-induced cell death; however, these agents have failed to show efficacy in clinical trials [7]. The need for alternative approaches to the treatment of stroke is therefore crucial.

Following stroke, patients suffer from a number of disabilities including motor impairments, cognitive decline, depression and anxiety [1,5,8,15,16,23]. An alternative approach for the treatment of stroke would therefore be to provide symptomatic relief from any one of these debilitating symptoms, resulting from a cerebral ischaemic event. At present, the majority of pre-clinical models of stroke have been fully characterised with respect to motor function, as this is initially the most disabling consequence of cerebral

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ischaemia. However, using current behavioural paradigms, such as neurological assessment or simple locomotor activity tests, many of the spontaneous motor deficits detected in animal models of stroke are shown to recover, over time, through recovery of function or adaptation. Although reflective of the clinical situation, this proves problematic for the evaluation of agents directed towards enhancing functional recovery [10,11,13,14,21]. The need to develop more sensitive/quantitative measures of motor function is, therefore, imperative. In addition, as patients are frequently able to adapt to their motor disabilities, it is the other aspects of stroke that can dominate recovery from an ischaemic event [1,23,25]. Affective disorders, such as anxiety and depression, have been reported to have an incidence as high as 28% in the post-stroke population, influencing both the severity and course of recovery from stroke. However, limited pre-clinical investigation has been undertaken to determine whether such incidence is reflected in the current animal models of stroke.

The present study was therefore designed to investigate the utility of a recently developed and validated automatic behavioural classification system Laboratory Animal Behavioural Observation, Registration and Analysis System (LABORAS), in assessing long-term functional outcome in a tMCAO model of stroke [17,18,26,29]. Although LABORAS is not capable of measuring fine motor function, the system offers many advantages over widely used subjective neurological scores as it can register home cage behaviour, which is more akin to quality of daily living scores utilised clinically. LABORAS registers the duration and frequency of feeding, drinking, grooming, rearing, locomotor activity (LMA) and immobility behaviours of a rat, individually housed in a home cage environment (free access to food and water), over a 24-h activity period for up to 7 days. The LABORAS system achieves this by transposing the mechanical vibrations caused by the movement of the experimental animal into electrical signals, which are scored and distinguished by a computer into the various behavioural categories, in accordance with unique amplitude and frequency patterns [22]. The social interaction paradigm, a test routinely used to assess anxiolytic/antidepressant agents [4,6,12], was also used to investigate the effect of ischaemia on rat social behaviour.

2. Materials and methods

All experiments were conducted according to the requirements of the United Kingdom Animals (Scientific Procedures) Act (1986) and conformed to GlaxoSmithKline Pharmaceuticals ethical standards.

2.1. Animals

Male Sprague–Dawley rats (Charles River, UK) weighing 300–350 g at the time of surgery were used. Prior to surgery animals were housed in groups of five, in a temperature-controlled environment (20±1 °C) and maintained with ad libitum access to food and water on a 12-h light/dark cycle (lights on 0700–1900 h). After surgery, rats were housed individually on soft bedding. Animals received fluids as required and were given free access to soft diet and baby food until pre-operative weight was regained.

2.2. Middle cerebral artery (MCA) occlusion

Transient (90 min) focal cerebral ischaemia was induced in rats. Under halothane anaesthesia, middle cerebral artery occlusion (MCAO) was carried out using the intraluminal thread technique as described previously [29]. Briefly, the left carotid arteries were exposed through a midline cervical incision and the pterygopalatine artery exposed and ligated at its origin with fine silk (5/0) leaving the extracranial carotid circulation contiguous. The external carotid artery was divided using diathermy forceps leaving a stump of ~2–3 mm in length and a small incision made to allow the advancement of a 3/0 gauge monofilament nylon suture coated in 0.1% poly-L-lysine (heat-blunted at the tip to achieve a diameter of 0.28–0.3 mm) from the left external carotid artery to the internal carotid artery up to the origin of the MCA. Sham animals underwent the same surgical procedure; however, the filament was not advanced into the MCA. Animals were maintained normothermic throughout the surgical procedure. Only those animals displaying tight circling behaviour 1 h post-MCAO were included in the study.

2.3. Assessment of lesion volume

Animals were maintained for up to 7 weeks, at which time they were killed by transcardial perfusion of 0.9% saline followed by 4% paraformaldehyde in 100 mM phosphate buffer. The brains were post-fixed in 4% paraformaldehyde at 4 °C for 48 h, at which time they were removed from the skulls and cut into 2 mm blocks using a rat brain matrix. The 2 mm sections were then paraffin-embedded using a Shandon Citadel 1000 tissue processor, cut into 6 μm sections using a microtome, mounted on poly-L-lysine-coated slides and processed for haematoxylin and eosin staining. Due to the degree of cavitation present at 7 weeks, the area of lesioned tissue was calculated by measuring the area of tissue remaining in the ipsilateral hemisphere (using an Optimas 6.1 imaging package) and subtracting this from the area of the contralateral hemisphere. The total lesion volume was expressed as % of the contralateral hemispheric volume calculated from the area measurements taken at seven predetermined levels 2 mm apart. The operator was blinded to treatment groups. Data are expressed as the total lesion volume (% of the contralateral hemispheric volume)±S.D., n=10–11.

2.4. Behavioural tests

Two weeks following tMCAO or sham surgery, rats were placed into the LABORAS system for a 24-h test period and data captured. Weekly thereafter, rats were placed into LABORAS cages overnight (1800–0700 h) and data again captured. Neurological score was assessed weekly prior to animals being placed in LABORAS. Social interaction behaviour was also assessed weekly, 2 days after LABORAS assessment.

2.4.1. Neurological score

One and twenty-four hours following surgery (and weekly thereafter), animals underwent neurological assessment as described previously [10], with the following modifications: grip strength measurements (scores 0–3); motility 0–4; general condition 0–4; and circling 0–5. Total score=33 for a normal animal. Data are presented as median scores, n=10–11. Statistical analysis was Mann–Whitney U-test at each time point measured.

2.4.2. Homecage behaviour

The LABORAS™ system (Metris, Hoofddorp, The Netherlands) consists of a triangular shaped sensing platform (Carbon Fibre Plate 700×700×1000×30 mm, Metris), positioned on two orthogonally placed force transducers (Single Point Load Cells) and a third fixed point attached to a heavy bottom plate (Corian Plate 695×695×980×48 mm, Metris). The whole structure stands on three spikes, which are adjustable in height and absorb external vibrations. Rats are housed in clear polycarbonate/Makrolon type III cages (floor area 840 cm^2, height 25 cm/height to food hopper 15 cm, cage: UNO Roestvaststaal, Zevenaar, The Netherlands, hopper and bottle: LabProducts, Seaford, USA), with a sawdust covered floor. One cage is placed directly onto the sensing platform, the upper part of which (including the top, food hopper and drinking bottle) is suspended in a high adjustable frame and is free from the sensing platform. Resultant electrical signals caused by the mechanical vibrations of the movement of the animal are transformed by each force transducer, amplified to a fixed signal range, filtered to eliminate noise, digitised and then stored on a computer. The computer then processes the stored data using several signal analysis techniques to classify the signals into the behavioural categories of feeding, drinking, rearing, climbing, immobility, LMA and grooming (for details, see Refs. [26,18]). The behaviour that dominates is scored. LABORAS data was captured as duration (seconds) and frequency for each behaviour. Data are presented as mean±S.E.M., n=10–11. tMCAO effects were analysed by two-way ANOVA with repeated measures.

2.4.3. Social interaction studies

The social interaction test was performed as previously described [12]. Briefly, rats were allocated to a test pair on the basis of weight (±10 g) and both were from the same surgery group. The extent of the social interaction was determined in a test arena constructed from black Perspex (54 (width)×36 (depth)×29 (height) cm), with a solid floor (divided into 24 squares, 9×9 cm) and a transparent front to allow observation of rat interaction by a camera mounted above, and to the front of, the arena. All testing was done under red light conditions. The behaviour of pairs of rats was videotaped by remote VCR in a room adjacent to that in which behavioural testing was conducted over a 15-min test session. All behaviour was performed between 0900 and 1700 h sequentially in blocked behavioural groups and all rats were habituated to the test arena 2 days prior to testing, for a 10-min period. The duration of active social interaction (interaction time), defined as grooming, following, sniffing, boxing, climbing under and over another rat; and locomotion (zone transitions; number of squares traversed on the base of the arena) was scored for each rat by an observer blind to experimental treatment. Data are presented as mean±S.E.M. for both sham and tMCA occluded groups, n=8 (4 pairs). tMCAO effects were analysed by two-way ANOVA with repeated measures.

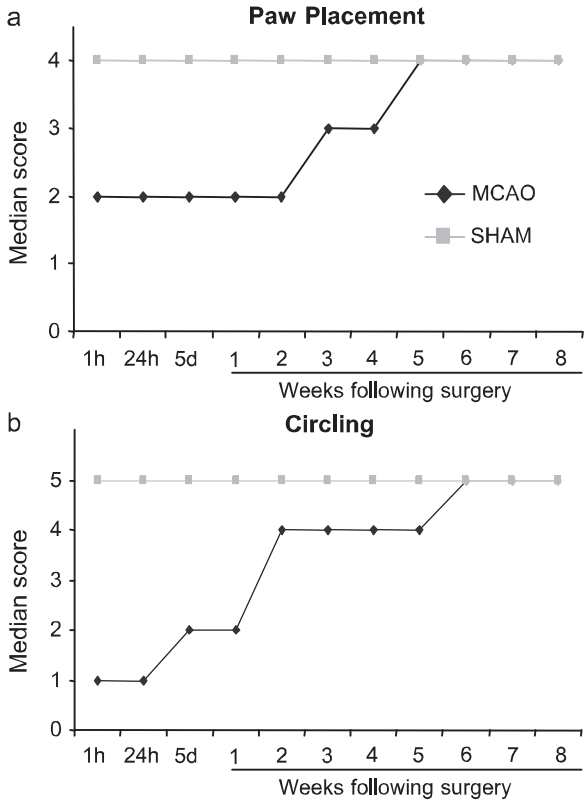

Fig. 1. (a) The score for paw placement, a constituent of the neurological score, decreases following tMCAO but recovers to sham levels by week 5. (b) The score for circling, a constituent of the neurological score, decreases following tMCAO but recovers to sham levels by week 6. All data cited as median values, n=10–11. *P<0.05 versus tMCAO animals, Statistical analysis was Mann–Whitney U-test at each time point measured.

3. Results

Histopathology: No ischaemic damage was detected in sham operated animals. In the MCAO group, total lesion volume was 29.9±8.8% (expressed as a percentage of the contralateral hemispheric volume). Ischaemic damage was detected throughout the parietal cortex and basal ganglia.

Weight loss: In the first week following MCAO, rats lose an average of 10–15% of their pre-operative body weight. The rats then gain weight progressively over subsequent

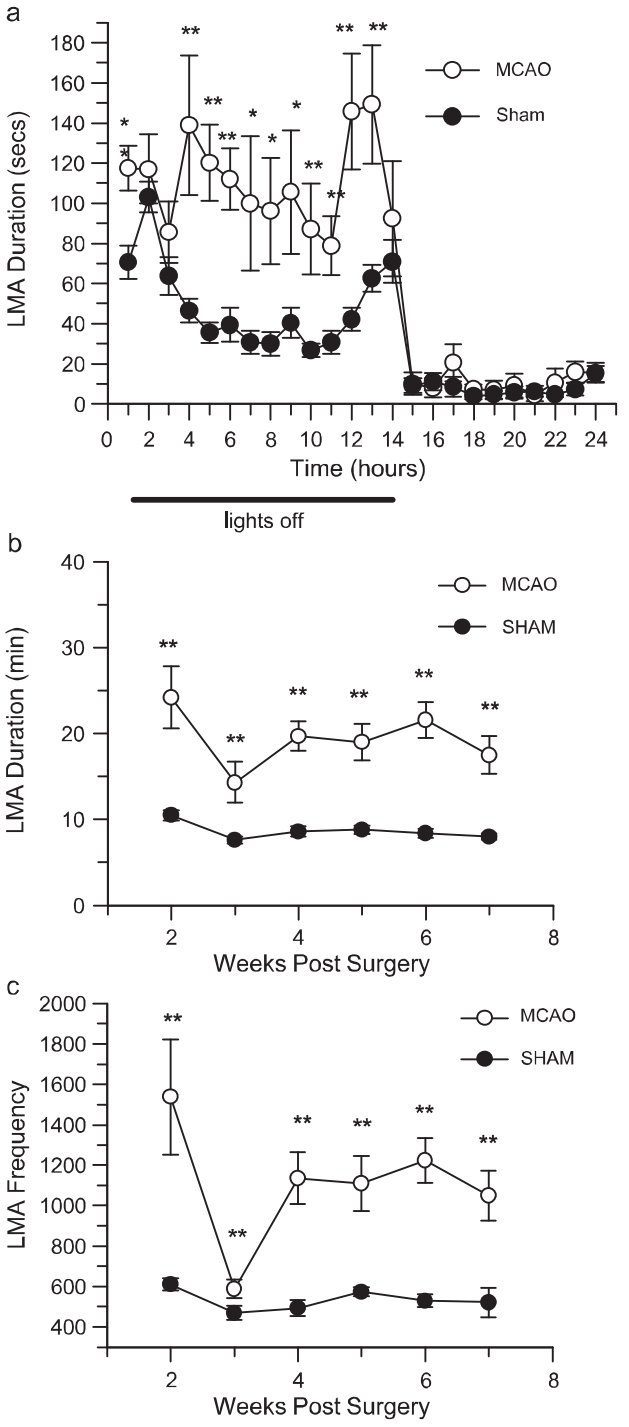

Fig. 2. (a) tMCAO-induced increase in LMA duration in rats over the first 24 h. (b) tMCAO-induced increase in LMA duration in rats, overnight. (c) tMCAO-induced increase in LMA frequency in rats, overnight. All data cited as mean±S.E.M., n=8–11/group. *P<0.05, **P<0.01—significantly different from sham operated controls, by two-way ANOVA with repeated measures.

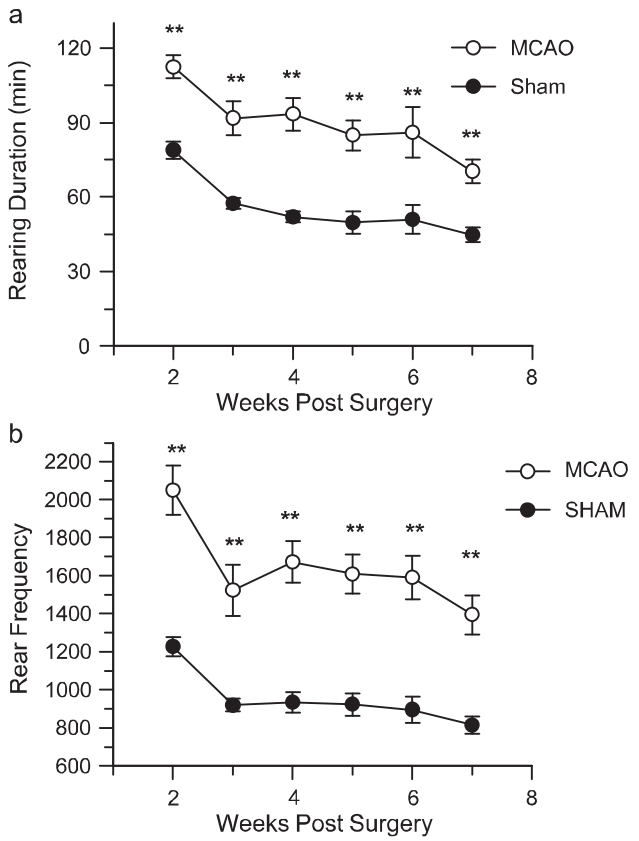

Fig. 3. (a) tMCAO-induced increase in rearing duration in rats, overnight. (b) tMCAO-induced increase in rearing frequency in rats, overnight. All data cited as mean±S.E.M., n=8–11/group. **P<0.01—significantly different from sham operated controls, by two-way ANOVA with repeated measures.

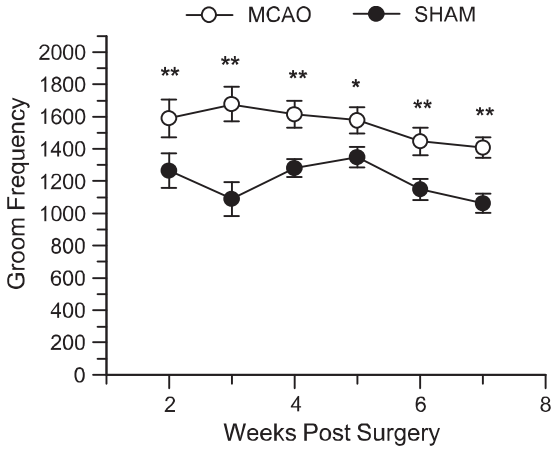

Fig. 4. tMCAO-induced increase in groom frequency in rats, overnight. All data cited as mean±S.E.M., n=8–11/group. *P<0.05, **P<0.01—significantly different from sham operated controls, by two-way ANOVA with repeated measures.

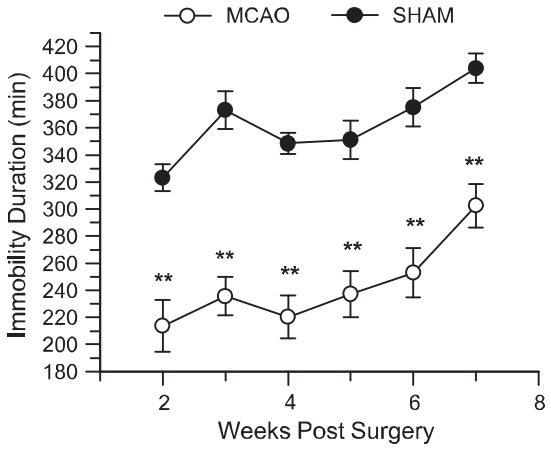

Fig. 5. tMCAO-induced decrease in immobility duration in rats, overnight. All data cited as mean±S.E.M., n=8–11/group. **P<0.01—significantly different from sham operated controls, by two-way ANOVA with repeated measures.

weeks, returning to their pre-operative weight by the end of the second week.

Neurological assessment: Sham animals exhibited a median total neurological score of 28 at 1 and 24 h following surgery. However, scores had reached 30 by week 1 and remained consistently high throughout the study. tMCAO animals exhibited a total neurological score of 16 at 1 h after occlusion, a score which rose modestly to 20 at 1 week, reaching 25 by week 3. Similar scores were maintained in the tMCAO animals throughout the study, the maximum score of 27 being achieved by week 6. The scores for paw placement and circling demonstrate that in these aspects of the test although tMCAO animals display a reduced score in the initial weeks of the study, by weeks 5 and 6, respectively, they obtained scores equal to that of sham animals (Fig. 1a,b).

3.1. Investigation of home cage and social behaviour in sham versus tMCAO rats

Home cage behaviour: LABORAS detected a highly statistically significant increase in nocturnal LMA duration (P<0.001 over time; Fig. 2a,b) and frequency (P<0.001 over time; Fig. 2c), rearing duration (P<0.001 over time; Fig. 3a) and frequency (P<0.001 over time; Fig. 3b) and groom frequency (P<0.05–0.001 over time; Fig. 4), and a corresponding decrease in immobility duration (P<0.01 over time; Fig. 5) in tMCAO animals, weeks 2–7 post

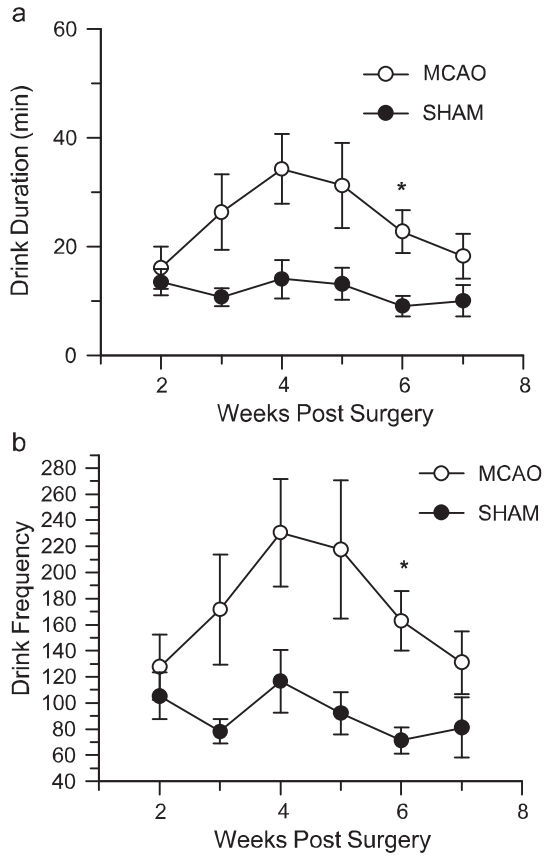

Fig. 6. (a) tMCAO-induced increase in drink duration in rats, overnight. (b) tMCAO-induced increase in drink frequency in rats, overnight. All data cited as mean±S.E.M., n=8–11/group. *P<0.05—significantly different from sham operated controls, by two-way ANOVA with repeated measures.

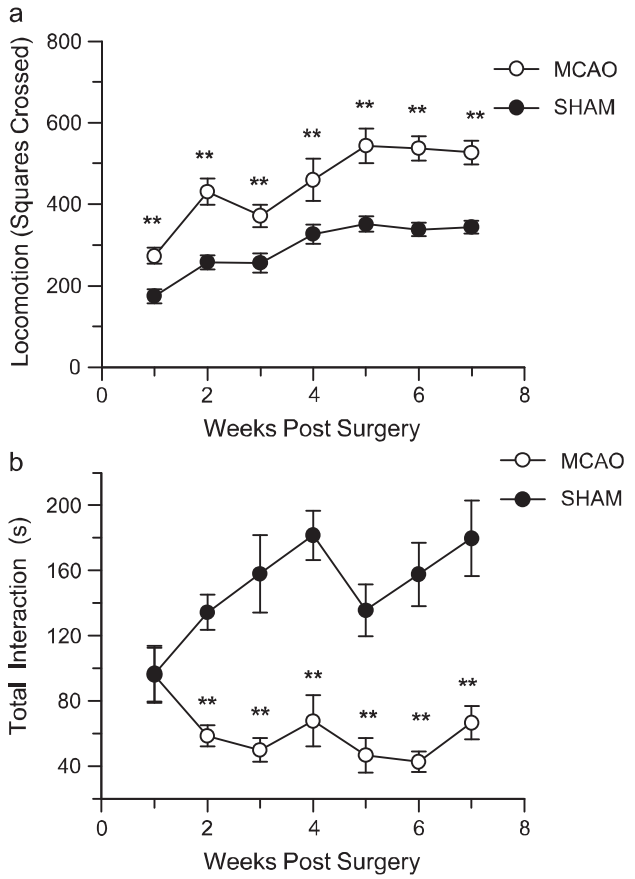

Fig. 7. (a) tMCAO-induced increase in spontaneous LMA in rats, in social interaction paradigm. (b) tMCAO-induced decrease in social interaction in rats, in social interaction paradigm. All data cited as mean±S.E.M., n=8–11/group. **P<0.01—significantly different from sham operated controls, by two-way ANOVA with repeated measures.

occlusion, when compared with shams. In addition, an increase in drinking and eating activity were also detected in tMCAO animals by LABORAS; however, statistical significance was only achieved at week 6 for drinking duration ($P<0.02$; Fig. 6a) and frequency ($P<0.02$; Fig. 6b), whereas eating duration and frequency did not reach significance (data not shown).

Social interaction and LMA: The social interaction test also detected a significant increase in spontaneous locomotor activity in tMCAO rats as early as 1 week following MCAO, which was maintained over time compared to controls ($P<0.05$–0.001 over time; Fig. 7a). In contrast, there was a significant decrease in the time spent in active social interaction from 2 to 7 weeks ($P<0.001$ over time; Fig. 7b) following MCAO, compared with sham operated controls.

4. Discussion

As previously reported, tMCAO animals showed marked reductions in neurological score compared with sham animals 24 h following surgery, which then gradually improved over time to the point where key aspects of the neurological test such as circling and paw placement returned to sham levels by 6 weeks [10]. LABORAS, however, detected a highly significant increase in nocturnal locomotor, rearing, grooming and drinking activities in tMCA lesioned animals, which persisted for up to 7 weeks after surgery, with no evidence of spontaneous recovery. The hyperactivity, as detected by LABORAS, is comparable with a study in which the nocturnal activities (1800–0600 h) of tMCAO occluded rats (60 min) were measured in a photobeam LMA system [2]. Indeed, both the present study and that of Borlongan et al. [2] noted tMCAO lesioned rats to have potentiated locomotor and rearing durations, when compared with sham operated controls at 1 and 2 months post-ischaemia. The hyperactivity detected by LABORAS is further supported by the significant potentiation of spontaneous LMA observed in the tMCAO lesioned rats in the social interaction arena. Moreover, permanent ligation of the right MCA in the rat has also been observed by some investigators to result in increased locomotor and rearing activity, when measured in the open field test [20]. These data are in contrast to previously reported investigations, where no effects on LMA have been observed between the control group and animals subjected to either transient [21] or permanent [27,28] MCAO. However, these authors used different methods of MCA occlusion and behavioural investigation to that described in the current study. Such contrast in reported findings further highlights the caution required when comparing data from different groups and the necessity to control for parameters such as time of test (daytime versus nighttime) and duration of test period.

The potentiation of motor activity observed in rats post MCAO has been hypothesised to be the result of striatal and cortical lesions of the monaminergic system, leading to the depletion and imbalance of monoamine transmission [20]. The hyperactivity observed in the present tMCAO model is the possible (functional) outcome of compensatory mechanisms initiated in surviving neurons in response to the dennervation of monaminergic pathways. Such compensatory mechanisms could include increases in neurotransmitter turnover, or postsynaptic receptor upregulation and supersensitivity. Indeed, it has been observed that on administration of the dopamine-releasing agent amphetamine, tMCAO rats are significantly more hyperactive than vehicle controls [2]. In addition, both the noradrenaline uptake blocker, imipramine, and the dopaminergic neurotoxin, 6-hydroxydopamine, block the development of post-infarction hyperactivity [19]. However, the participation of injured non-catecholaminergic neurons cannot be ruled out [2,20]. Nevertheless, substantial damage was observed in both the striatum and the cortex, which suggests that the lesioning of pathways in these areas may be responsible for the behavioural effects observed.

In the present investigation, those animals that had undergone tMCAO surgery were also shown to have a significantly reduced duration of active social interaction compared with sham operated animals. This was noted by the observer to be the result of an ability to initiate but not maintain social contact. Moreover, tMCAO lesioned rats were observed to be extremely aggressive during the test period, indicative of a heightened anxiety-like state [6]. Previously, limited pre-clinical investigation has been carried out on the effects of experimental stroke on anxiety-like behaviours in the rat; however, Harukuni et al. [9] have reported that tMCA occlusion in rats results in impairments in the elevated plus maze paradigm.

In conclusion, the data described in the present study provides further evidence that long-term functional deficits following transient cerebral ischaemia in rats are demonstrable. Moreover, these behavioural deficits can be seen in LABORAS under home cage conditions in the absence of lengthy behavioural pre-training and pharmacological intervention. In addition, LABORAS has several advantages over conventional photobeam LMA systems. The duration and frequency of multiple behaviours are automatically scored in a single experiment and the use of a home cage environment allows studies of a much longer duration with continuous monitoring to be undertaken. However, it should be highlighted that LABORAS can only recognise behaviours that have established algorithms. Moreover, the LABORAS system can only be programmed to identify behaviours that produce vibrational signals of significant amplitude and consistency. More complex or refined behaviours such as yawning or disturbances of gait would therefore not be recognised.

LABORAS and the social interaction test therefore provide novel, highly sensitive, quantitative methods for the pre-clinical assessment of potential symptomatic stroke therapies.

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