RESEARCH REPORT

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Delayed citalopram administration reduces brain inflammation and enhances skilled motor function after ischaemic stroke in 'MacGreen' mice

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Abstract

Recent evidence suggests that treatment with antidepressants may promote functional recovery. However, the timeframe in which these pharmacological agents can influence stroke recovery is not well understood. This research investigated whether delayed administration of citalopram, used clinically in the management of post-stroke depression, could improve long-term functional recovery following experimental stroke. MacGreen mice carrying an enhanced green fluorescent protein reporter gene in monocyte and macrophage populations were subjected to 45 min occlusion of the middle cerebral artery. Animals were administered citalopram (10 mg/kg/day, $n = 20$) or saline ($n = 20$) starting 3 days after stroke for 28 days. Neurological deficits and skilled motor performance in the staircase task were recorded for 9 weeks post stroke. Grey and white matter structural lesions were quantified at Week 9, and enhanced green fluorescent protein immunohistochemistry was used to evaluate the effect of citalopram on inflammation. Twenty-five animals were included in the final analysis. Citalopram-treated animals ($n = 13$) showed a significant increase in impaired forepaw use in the staircase task compared with saline-treated animals ($n = 12$) 2, 3 and 7 weeks post stroke but no difference in neurological score at any time point examined. Citalopram treatment was associated with decreased monocyte/macrophage cell density and increased white matter tract integrity within the ipsilateral cortex. In conclusion, delayed administration of citalopram decreased brain inflammation and produced functional gains in our mouse model of stroke. Beneficial effects on skilled motor functions were long-lasting.

Abbreviations: ANOVA, analysis of variance; CNS, central nervous system; CSF-1R, CSF-1 receptor; MacGreen, CSF-1R-EGFP transgenic mouse line; EGFP, enhanced green fluorescence protein; M/M, macrophage/monocyte/microglia; MCA, middle cerebral artery; MCAo, middle cerebral artery occlusion.

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1 | INTRODUCTION

Stroke is ranked as the second leading cause of death worldwide and is a leading cause of disability (Donkor, 2018). There are over 25.7 million stroke survivors worldwide and over 50% suffer from long-lasting impairments that affect their ability to perform daily activities (Donkor, 2018). The social and economic consequences of providing chronic care for these patients will continue to increase unless new treatments to recover lost function are developed.

There is growing evidence that pharmacological enhancement of monoamine neurotransmission can markedly alter brain remodelling and behavioural recovery after stroke (Gu & Wang, 2018; Legg et al., 2019). While a degree of motor recovery may result from improved mood and motivation to perform rehabilitation tasks, experimental studies demonstrate that antidepressants, particularly selective serotonin reuptake inhibitors (SSRIs), directly affect brain remodelling and plasticity processes (Espinera et al., 2013; Schneider et al., 2019). More recently, there is modest evidence from the clinical setting that SSRIs promote recovery of function after stroke (Chollet et al., 2011; Jorge et al., 2003; Tallelli & Werring, 2009). However, the role that SSRIs could play in post-acute stroke care remains to be fully determined. While meta-analyses indicates SSRIs have generally favourable effects on gross motor function (Gu & Wang, 2018; Mead et al., 2013; Yeo et al., 2017), Legg et al. (2019) showed that inclusion of only trials at low risk of bias does not support routine use.

We propose that the heterogeneity between trials in timing, duration, drug and dosage underlies the variation in results. Pin-pointing the ideal timing and agent for therapeutic intervention is therefore of particular importance to maximise functional benefit in stroke patients (AFFINITY Trial Collaboration, 2020).

We propose that the multimodal effects of SSRIs may still hold promise as a disease-modifier in post-stroke recovery. Citalopram appears to have comparable efficacy to fluoxetine (Asadollahi et al., 2018) but has linear kinetics over its therapeutic dose range, and steady-state plasma concentrations can be achieved within a week of daily dosing. The improved pharmacokinetic profile and lower potential for drug interactions (Marken & Munro, 2000) suggests citalopram may have better clinical utility in promoting motor recovery after stroke than fluoxetine. The present study investigated whether administration of citalopram in the subacute phase (3 days) after stroke could promote long-lasting improvements in function in a clinically relevant mouse model of stroke.

2 | EXPERIMENTAL PROCEDURES

2.1 | Ethical statement

All animal work was carried out in accordance with the Animal Welfare Act 1999 and was preapproved by the University of Auckland Animal Ethics committee (Animal Ethics #R1245). This work adheres to RIGOR reporting guidelines (Lapchak et al., 2013).

2.2 | Animals

Founder MacGreen mice (strain of origin C57BL/6 × CBA F1) were gifted by the Queensland Brain Institute, University of Queensland, Australia. Generation of the strain has been described elsewhere (Chen et al., 2017; Chen & Bennet, 2015; Sasmono et al., 2003). The MacGreen colony was maintained as homozygote, and all offspring were positive for the transgene.

MacGreen (CSF1R-EGFP) mice carry an enhanced green fluorescent protein (EGFP) gene downstream of the c-fms promoter. The c-fms gene encodes the receptor for macrophage colony-stimulating factor (CSF-1) and is expressed selectively in macrophage and monocyte cell lineages (Sasmono et al., 2003). MacGreen mice provide a model system for the study of macrophage development and function in the periphery (Lückoff et al., 2017; Sasmono & Williams, 2012) and offer a unique opportunity to visualise and investigate brain inflammation post stroke in conjunction with functional outcomes (Chen & Bennet, 2015).

Male MacGreen mice (23–28 g) were housed in single sex cages in bioBUBBLE soft wall cleanrooms maintained at 20°C with a 12-h day and night cycle. Post-operatively, mice were group housed ($n = 5$–6 animals per cage). To maintain blinding for subsequent behavioural testing, treatment groups were not housed separately.

2.3 | Cerebral ischaemia

Animals were assigned to experimental groups (citalopram or saline, $n = 20$/group) using a randomisation table generated by Excel. Monofilament occlusion of the middle cerebral artery (MCA) was performed contralateral to the dominant limb of the animal. Dominance was based on performance during the final week of training for the staircase test. Mice showing indistinguishable performance between the two limbs received middle cerebral artery occlusion (MCAo) in the right hemisphere. The proportion of right and left MCAo was comparable between groups.

MCAo was performed under isoflurane anaesthesia as previously described (Chen et al., 2017; Chen & Bennet, 2015). Briefly, the common carotid (CCA) was exposed through a midline cervical incision, where an 8.0 silicone-coated monofilament was introduced and advanced 10 mm distal to the carotid bifurcation, occluding the origin of the MCA for 45 min. At the end of the occlusion period, the monofilament was removed and the surgical site sutured closed. Animals were recovered in a humidified incubator until freely moving and then returned to their home cages. Supplementary analgesia was provided by the addition of paracetamol (30 mg/ml) in drinking water 1 day prior to and for 3 days after stroke surgery.

2.4 | Drug administration

Mice were randomly assigned to receive daily intraperitoneal (ip) injections of citalopram HBr (10 mg/kg/day, Tocris, Bristol, UK, Cat: #1427) dissolved in .9% saline or saline alone, starting from day 3 post stroke for 28 consecutive days. Injections and functional testing were performed by different researchers.

2.5 | Neurological evaluation

The welfare of the animals was monitored daily postoperatively for the first week and then weekly thereafter. Animals were weighed, and their gross neurological deficit was assessed by an experimenter (SC) blinded to experimental condition using a 15-point neurological deficit score (NDS) adapted from a modified clinical assessment (Chen et al., 2017). This assessment examined general condition, mobility, spontaneous circling behaviour, righting reflex, forepaw reach, forepaw placement, posture readjustment on inclined platforms and movement when rotating by the base of the tail. Normal animals display an NDS of 15, while animals subjected to

MCAo typically have neurological scores ranging between 8 and 10 (Chen & Bennet, 2015).

2.6 | Staircase test

The staircase test was implemented to measure the skilled motor function. A detailed protocol has been previously described elsewhere (Bouet et al., 2007). Briefly, animals were subjected to food restriction throughout the training and testing period to provide motivation. Food restriction comprised access to standard lab chow for 4 h per day between 4.30 PM and 7.30 PM. Mice received 3 weeks of training prior to MCAo surgery in fully bait (Purified Dustless Precision Pellets® for Rodent, Able Scientific, Perth, Australia, Cat#: F0071) loaded testing apparatus (Campden Instruments, Loughborough, UK).

Dietary restriction was terminated at 4.30 PM on the last training day, and mice returned to ad libitum feeding for 3 days prior to MCAo surgery until 8.30 am on Day 6 after surgery.

Post-stroke testing began on Day 7 post stroke to avoid potential welfare complications associated with food restriction early after an ischaemic insult. Animals were subjected to one 10-min trial per testing day. Post-stroke testing was blocked by testing week. The first 2 days of each testing week were designated as retraining sessions and were not included in the final analysis. Performance in the final 3 days was averaged as a score for that testing week. Post-stroke testing of the staircase test was performed on Weeks 2, 3, 5, 7 and 9.

2.7 | Exclusion criteria

Forty animals were assigned to the study. Animals were excluded from the study if the performance in the staircase test did not reach the predesignated baseline performance level ($n = 2$), death occurred after stroke surgery ($n = 3$) or the animal did not survive until the end of the study ($n = 10$).

2.8 | Histological analyses

Sixty-three days after stroke surgery, animals were killed by trans-cardiac perfusion with 4% paraformaldehyde under deep anaesthesia (pentobarbitone, 60 mg/kg ip) and the brains removed and processed for quantitative histological analysis. Microtome sections (30 μm) were stained with thionin and examined for damage at nine stereotaxic levels by light microscopy. Image analysis (Neurolucida, MicroBrightField Inc., USA) was used to

determine ipsilateral and contralateral hemispheric volumes and the volume of damage in animals by an observer blinded to the experimental condition.

Incidence maps showing the patterns of ischaemic damage at the level of the striatum (Bregma +.98) were generated as described previously for animals with frank lesions (Chen et al., 2017). The rate of occurrence of histological damage occurring in specific regions was then indicated by colour (regions with histological damage in 25% to 49% of the animals were coloured light grey; 50% to 74% of animals, dark grey; 75% to 100% of animals, black).

BlackGoldII (Millipore, CAT#: MPAG400, USA) was used to visualise white matter in animals with frank lesions (Schmued et al., 2008). Tile scan images of cross section brain at Bregma +.98 mm and Bregma +1.54 mm were acquired (Nikon TE2000E Inverted Microscope) for qualitative and quantitative analyses of white matter tracts in the striatum (ImageJ, Rasband, NIH, USA).

2.9 | Immunohistochemistry

Adjacent free-floating sections from mice in each group were taken for single- and double-label immunohistochemistry as previously described (Abbate et al., 2015; Chen et al., 2017). EGFP transgene expression was enhanced using an antibody to green fluorescence protein (GFP) (rabbit anti-GFP, 1:5000, CAT#: AB290, AbCam®, UK Abcam, Cambridge, UK). Volumetric analysis was performed to quantify the total volume of tissue with EGFP expression, relative to contralateral

hemisphere, and incidence maps at Bregma +.98 mm were generated.

The number of EGFP positive cells within the core and peri-infarct regions of the striatum and the cortex was assessed semiquantitatively. Two sections from each animal at Bregma −0.1 mm was analysed at 20× magnification. Three images were taken from predefined areas (1.2 mm²) and the number of EGFP positive cell bodies counted manually to determine an estimated cell density per square millimetre.

The signal intensity of EGFP immunofluorescence was estimated by converting the acquired images to 8-bit grey scale. A thresholding function was manually defined using ImageJ, and the relative area displaying increased EGFP expression, integrated optical density and mean area intensity were measured using ImageJ to acquire fluorescence unit per square millimetre. Confocal microscopy images were acquired using an Olympus FV1000 confocal microscope at excitation wavelength 488 nm.

2.10 | Statistical analysis

Power analysis from historical data indicated that 10 animals per group were required to detect a 10% difference in skilled motor performance with 80% confidence.

The randomisation code was broken after all data were acquired to allow allocation to experimental groups. All statistical analyses were carried out using SPSS (PASW Statistics 18, 2009). Comparison of survival was analysed using Kaplan–Meier survival function on SPSS. NDSs were analysed using Friedman one-way repeated measures ANOVA followed by Mann–Whitney rank-sum
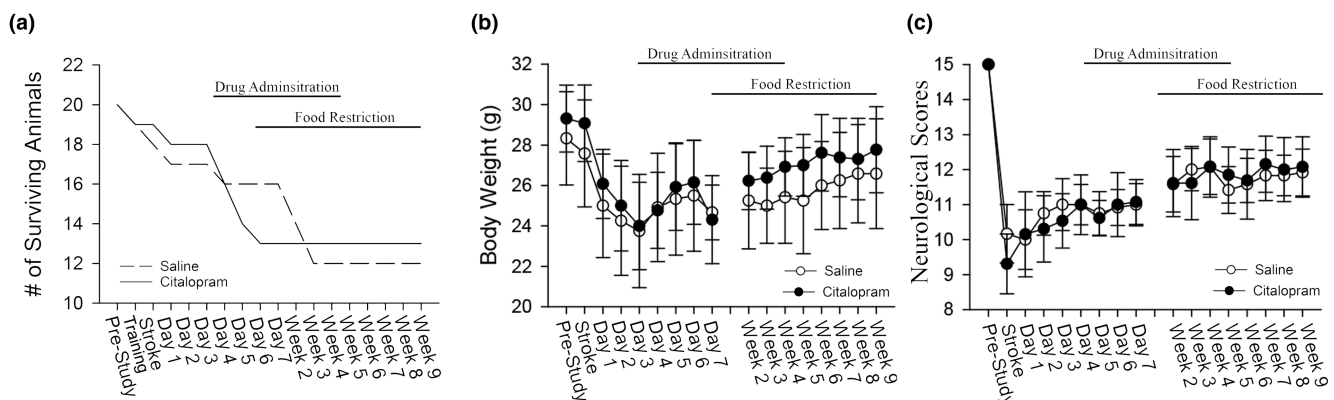

FIGURE 1 Welfare monitoring of citalopram- and saline-treated animals during the study. (a) Kaplan–Meier plot showing survival of saline- and citalopram-treated animals was comparable over time ($p = .951$). (b) Stroke surgery significantly reduced body weight over the first 3 days post stroke ($p = .001$). Citalopram-treated animals had significantly higher body weight at Weeks 5 ($p = .029$) and 6 ($p = .018$) post stroke. (c) Ischaemic injury resulted in immediate impairments in neurological score in all animals ($p = .001$). Saline- and citalopram-treated animals had comparable patterns of recovery in neurological scores throughout the study ($p = .726$). Data are presented as mean ± SEM, $n = 12$–13 per group

t tests. Two-way ANOVA was used to assess differences in weight and skilled motor function. Experimental data for histological and immunohistological analyses were analysed using two-way ANOVA with Bonferroni test for pairwise comparisons. Values presented in this study are mean ± standard deviation. Statistical significance was set at $p < .05$.

3 | RESULTS

3.1 | Weight and neurological deficits

Figure 1a shows the assignment and fate of animals in this study. Comparable rates of survival were found in both treatment groups ($p = .951$, $t = .004$). A total of 25 animals (saline $n = 12$, citalopram $n = 13$) were included in the final analysis with an overall exclusion rate of 37.5%. Significant weight loss was observed in both groups over the first 3 days after stroke ($p = .001$, $t = 13.021$, Figure 1b) and at Day 7 when food restriction was reinitiated ($p = .04$, $t = 3.527$). Citalopram-treated animals had significantly higher body weights than control animals on Day 35 ($p = .029$, $t = 1.995$) and Day 42 ($p = .018$, $t = 2.232$) post stroke. MCAo surgery was associated with neurological deficits in all animals immediately after stroke ($9.74 \pm .15$, $p = .001$, $t = 5.263$, Figure 1c). Comparable changes in neurological score were observed between both treatment groups following stroke ($p = .726$, $t = 5.033$)

3.2 | Motor function recovery

The staircase test was used to examine skilled motor function in all animals. There was no significant difference in the average number of pellets consumed ($F_{(1,149)} = .37$, $p = .549$), displaced ($F_{(1,149)} = .0513$, $p = .823$) and the maximum depth of step reached ($F_{(1,149)} = .135$, $p = .717$) by unimpaired limbs (Figure S1).

MCAo resulted in a significant reduction in the average number of food pellets retrieved by all animals ($p = .001$, $t = 4.47$, Figure 2a). However, citalopram-treated animals consumed significantly more pellets than saline-treated animals at Week 2 ($p = .041$, $t = 1177$) and Week 3 post stroke ($p = .010$, $t = 1131.5$). While the number of food pellets retrieved was not different between the two treatment groups at 5 weeks after stroke ($p = .293$, $t = 1270$), statistical significance was again observed 2 weeks after citalopram treated stopped (Week 7 post stroke; $p = .021$, $t = 1156$) but not after 4 weeks of washout (Week 9 post stroke; $p = .905$). A comparable
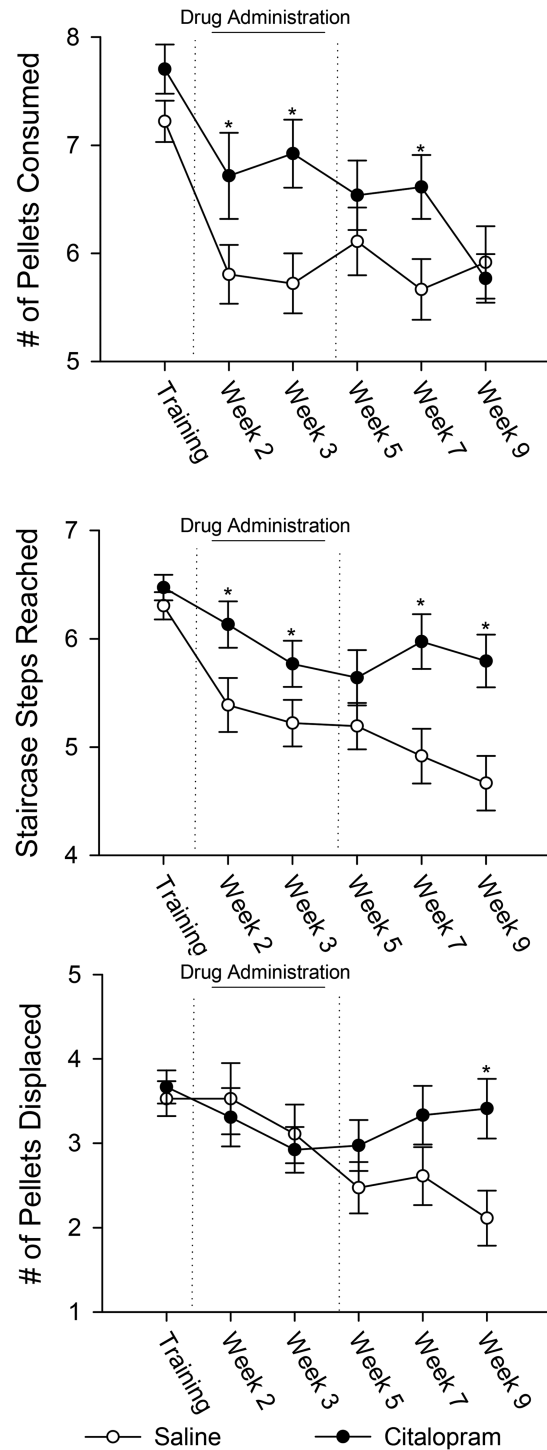

FIGURE 2 Skilled motor function of the impaired forelimb. (a) Citalopram significantly increased the number of food pellets retrieved by the impaired limbs at 2 ($p = .041$, $t = 1177$), 3 ($p = .010$, $t = 1131.5$) and 7 ($p = .021$, $t = 1156$) weeks after stroke. (b) Citalopram-treated animals were able to reach further on Weeks 7 ($p = .034$, $t = 2.192$) and 9 ($p = .024$, $t = 2.338$) after stroke compared with saline-treated animals. (c) Citalopram-treated animals displaced a significantly higher number of food pellets 9 weeks after stroke compared with those by saline-treated animals ($p = .045$, $t = 2.044$). Data are presented as mean ± *SEM*, $n = 12$–13 per group

number of pellets were displaced between the two treatment groups for the first 6 weeks after stroke. Citalopram-treated animals displaced more food pellets than saline-treated animals 9 weeks after stroke ($p = .045$; $t = 2.044$, Figure 2c).

MCAo significantly reduced the depth of step reached by saline-treated animals ($p = .014$, $t = 3.302$, Figure 2b). However, citalopram-treated animals were able to reach significantly further on Weeks 7 ($p = .034$, $t = 2.192$) and 9 ($p < .024$, $t = 2.338$) after stroke compared with saline-treated animals.

3.3 | Grey matter lesion volume

Qualitative examination of thionin-stained sections showed MCAo-induced damage in the striatum and cortex of animals of both treatment groups. Damage could be observed as a frank histological lesion (observable as an area of pallor) or as a densely stained resolved lesion. Incidence maps created for animals with overt lesions showed a higher frequency of cortical damage in saline-treated animals; all saline-treated animals had lesion in the cortex (Figure 3a) while 50% of citalopram-treated animals had cortical lesions (Figure 3b). Quantitative analyses of thionin histology showed no statistical differences in lesion volume or distribution between the treatment groups at 63 days post stroke ($p = .245$, $t = .744$,

Figure 3c). Ipsilateral tissue atrophy (% tissue loss) was calculated for animals with no overt lesion. Tissue loss between treatment groups was not statistically significant ($p = .073$, $t = 1.529$, Figure 3d).

3.4 | EGFP expression

Regions of increased EGFP expression in animals with overt histological lesions broadly correlated with the pattern of ischaemic damage. While the extent of the inflammatory response was comparable ($p = .486$, $t = .036$, Figure 4), qualitative analysis of incidence maps generated from tile scan images showed a higher incidence of increased EGFP expression in the cortex of saline-treated animals (Figure 4a).

Quantitative analyses of EGFP expression showed a significant reduction in both EGFP signal intensity ($p = .039$, $t = 1.797$, Figure 4d) and the density of EGFP positive cells ($p = .033$, $t = 3.934$, Figure 4c) in the cortex of citalopram-treated animals compared with saline-treated animals.

3.5 | White matter preservation

Qualitative examination of BlackGoldII staining in animals with overt grey matter lesions showed
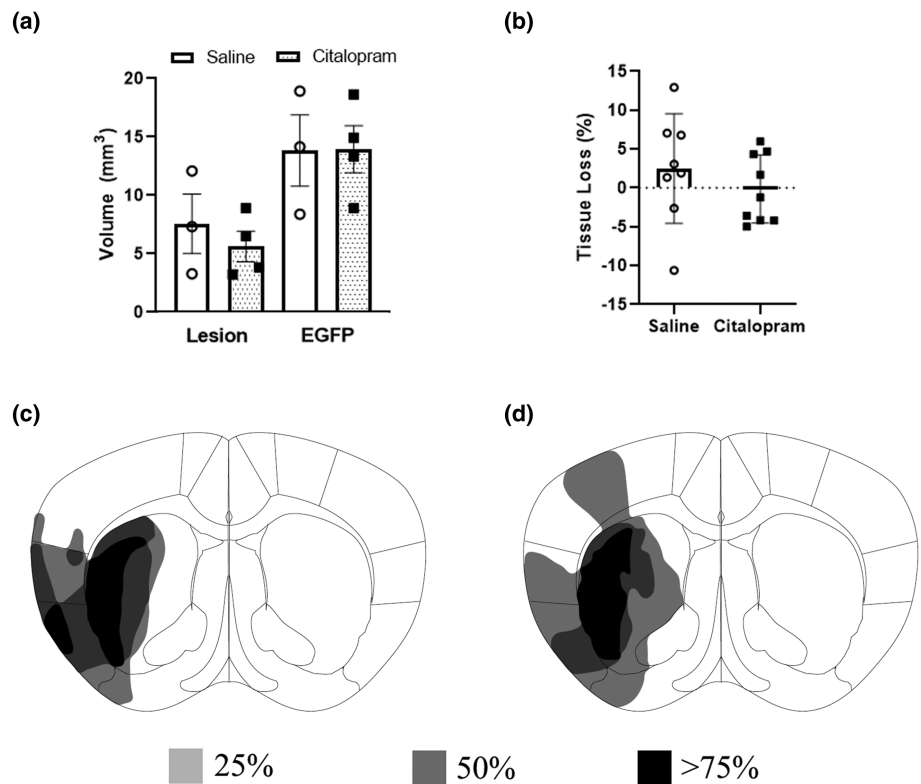

FIGURE 3 Lesion distribution, volume and tissue loss in saline- and citalopram-treated animals. Quantitative analyses showed comparable lesion volumes in animals with frank infarcts (a; $p = .245$), data are presented as mean $\pm$ SEM, $n = 3$–4 per group. The volume of tissue loss in the ipsilateral hemisphere of animals with resolved lesions at 63 days was also comparable (b; $p = .073$, $n = 8$–9 per group). Incidence maps showing patterns of ischaemic damage in (c) saline- and (d) citalopram-treated animals with frank infarcts at 63 days. Regions with histological damage in 25% to 49% of the animals were coloured light grey; 50% to 74% of animals, dark grey; 75% to 100% of animals, black
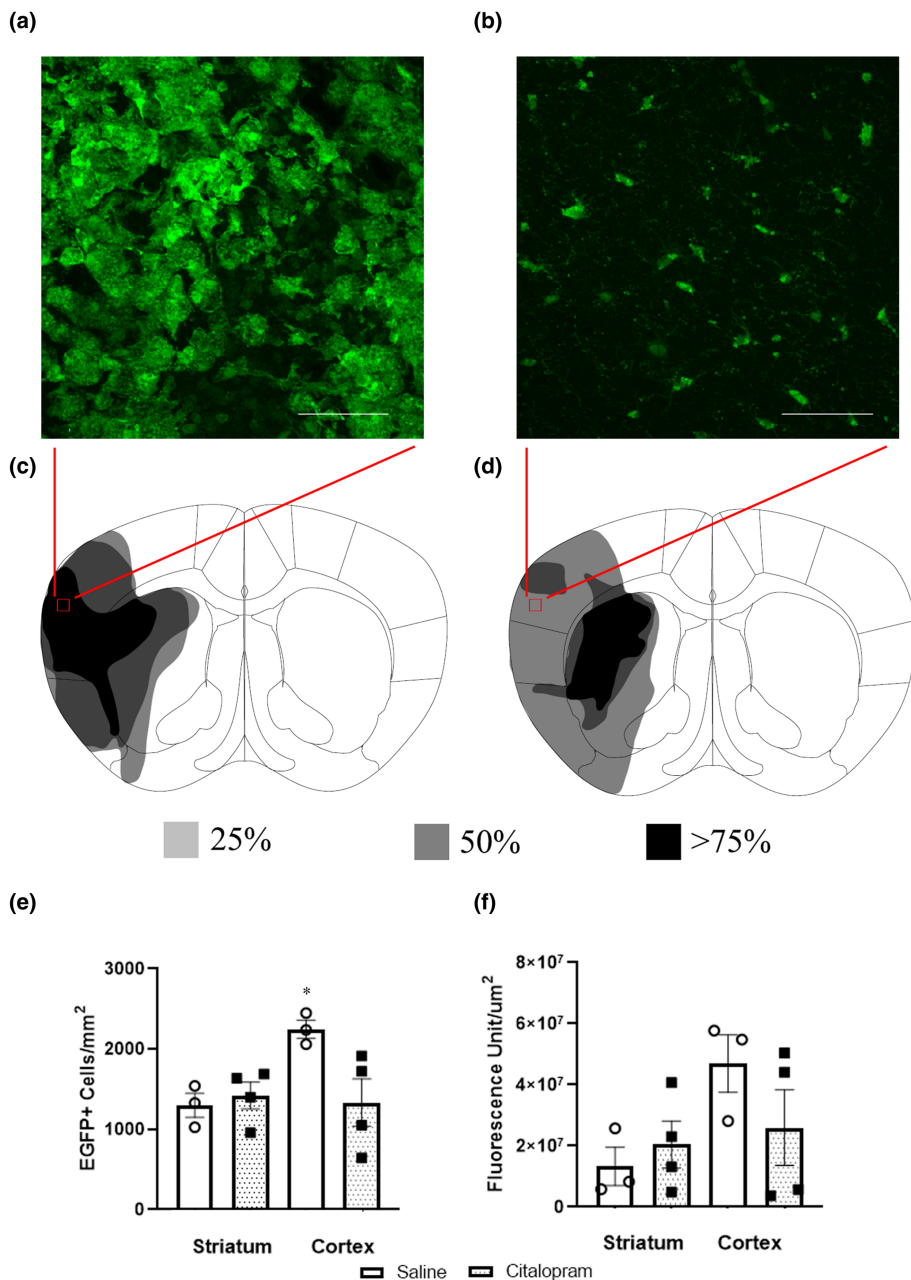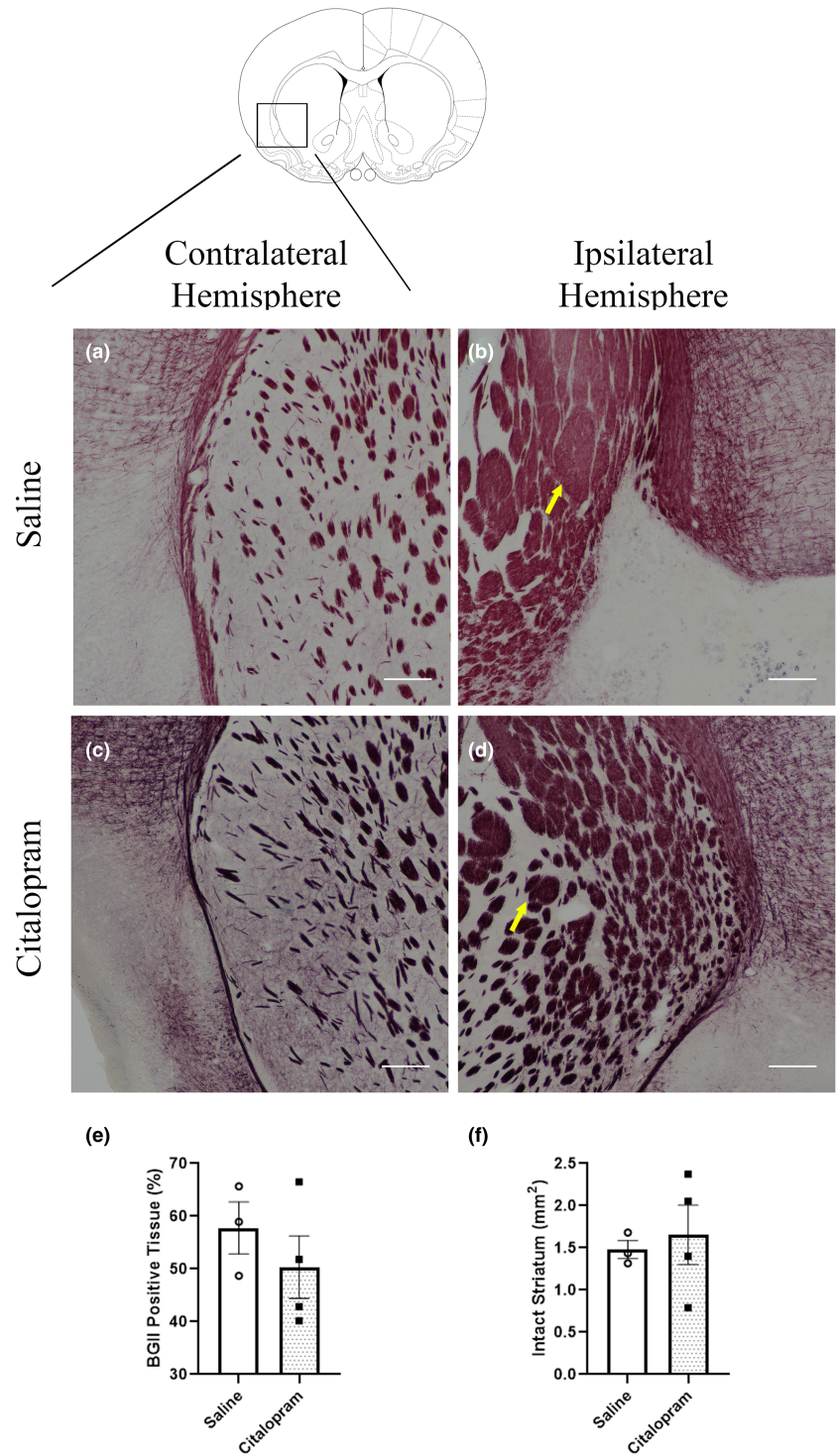
(a)

(b)

(c)

(d)

| 25% | 50% | >75% |

(e)

(f)

FIGURE 4 EGFP expression in saline- and citalopram-treated animals. Representative confocal images from the cortex of (a) saline- and (b) citalopram-treated showing EGFP expression in animals with frank infarcts at 63 days. Scale bar = 50 μm. Incidence maps showing patterns of EGFP expression in (a) saline- and (b) citalopram-treated animals. Regions with histological damage in 25% to 49% of the animals were coloured light grey; 50% to 74% of animals, dark grey; 75% to 100% of animals, black. A significant reduction in the number of EGFP positive cells (c; $p = .033$, $t = 2.535$) and signal intensity of EGFP expression (d; $p = .039$, $t = 1.261$) was found in the cortex of citalopram-treated animals. Data are presented as mean $\pm$ SEM, $n = 3–4$ per group

demyelination and axon swelling in ipsilateral hemispheres (Figure 5b,d) compared with that of the contralateral hemispheres (Figure 5a,c). Citalopram treatment appeared to attenuate the ischaemia-induced demyelination and axon swelling in the ipsilateral hemisphere (Figure 5d) compared with that of saline-treated animals (Figure 5b). Quantitative analyses confirmed a significantly larger proportion of axon swelling in saline compared with citalopram-treated animals (57.79% $\pm$ 5.63% and 42.75% $\pm$ 4.34%, respectively, $p = .028$, $t = 2.052$; Figure 5e). The corresponding difference in demyelination just failed to reach statistical significance (2.29 $\pm$ .33 and 1.48 $\pm$ .08 mm^2, respectively, $p = .056$, $t = 1.685$; Figure 5f).

4 | DISCUSSION

This study showed for the first time that citalopram administration, delayed for 3 days, was associated with improved skilled motor function performance in MacGreen (CSF-1R-EGFP) mice following experimental stroke. Improved performance was sustained for 2 weeks after drug administration stopped and associated with reduced inflammation and preservation of white matter structural integrity but no reduction in lesion size. Our results extend the timeframe for SSRI administration proposed by Schneider et al. (2019) and provide evidence that suppression of inflammation contributes to the multitarget directed effects of SSRIs reported by others (Acler

et al., 2009; Dhami et al., 2013; Espinera et al., 2013; Schmidt & Duman, 2007).

MacGreen mice carry an EGFP gene downstream of the c-fms promoter. The c-fms gene encodes the receptor for macrophage colony-stimulating factor (CSF-1) and is expressed selectively in macrophage and trophoblast cell lineages in the spleen, lung, intestine, brain and thymus (Sasmono et al., 2003). We have shown previously that EGFP expression is upregulated in the ipsilateral hemisphere of MacGreen mice post stroke (Chen & Bennet, 2015). EGFP positive cells colabelled with the microglia/macrophage marker Iba1 and changes in the morphology of these cells from 24 h to 35 days reflect temporal changes in the function of microglia/macrophages within ischaemic regions. Here, we show that EGFP expression remained upregulated

in animals with the most severe ischaemic damage out to 63 days after stroke. The observed reduction in both EGFP positive cell density and signal intensity in the cortex suggests that citalopram reduced monocyte/macrophage activation after severe stroke and adds weight to our overarching hypothesis that reducing inflammation in the subacute phase after stroke using pharmacological agents can promote functional recovery (Chen et al., 2017).

As with other brain injuries (Bennet et al., 2018; Lenzlinger et al., 2001) an early, well-controlled inflammatory response after stroke is beneficial and leads to healing (Nathan, 2002) while prolonged inflammation inhibits repair and negatively correlates with functional outcome (Moxon-Emre & Schlichter, 2010; Thiel et al., 2010). Strategies that reduce the initial stage of the inflammatory response have failed to show benefit. In keeping with this, acute administration of citalopram within the first hours after spinal cord injury or stroke decreased inflammatory cells but failed to improve motor performance (Gupta et al., 2018; Lima et al., 2020); however, administration 24 h after stroke improved short-term sensorimotor function in an experimental stroke model (Espinera et al., 2013).

Our report of long-term functional improvement following delayed administration confirms that counteracting the later phase of inflammation may be a more effective treatment strategy and that a window of opportunity for treatment may extend to at least 72 h post stroke. It remains unclear whether the intervention time window remains evident beyond this timeframe, although clinical studies show improved functional outcomes when citalopram is administered within 7 days of stroke (Asadollahi et al., 2018; Savadi Oskouie et al., 2017). Together with the observation of persistent functional benefit beyond treatment in the current study, the differences in treatment period in these clinical trials suggest it is 'when' citalopram is initiated in relation to stroke rather than the duration of treatment that is important. Nevertheless, studies investigating whether the apparent disease modifying effects support intermittent rather than continuous dosing are warranted to optimise clinical application.

The novel observation that citalopram reduced white matter injury suggests that inflammation-induced axonal damage occurring in the days and weeks after stroke is of functional relevance for recovery. Reducing microglial activation via pharmacological or physical means has been shown to improve white matter myelination and reduce cognitive dysfunction after experimental stroke (Jackson et al., 2020; Qin et al., 2017). Our results suggest a causal link also exists between activation of macrophage/microglial cells and the white matter damage that leads to motor dysfunction. Indeed, citalopram and its active s-isomer escitalopram can alter motor output (Weisstanner et al., 2018) and manual dexterity in chronic stroke patients after a single dose (Zittel et al., 2008), suggesting sensorimotor network level effects beyond direct protection of the neurovascular unit (Espinera et al., 2013). The functional benefits of citalopram appear related not to neuroprotection but neurorepair through alterations in cortical motor mapping that occur in the weeks after stroke (Pinto et al., 2017; Schneider et al., 2019).

The strengths of this study include assessing functional performance and brain inflammation in the same animals. Downstream analysis in animals with overt histological lesions at 9 weeks highlights the mechanisms contributing to functional improvements. Using MacGreen mice to visualise inflammation in stroke studies is novel and the inclusion of white matter histology strengthens our results. Our study has a few limitations applicable to preclinical research using disease models. It is increasingly apparent that the response to ischaemia differs in aged animals and in those with comorbidities (Ankolekar et al., 2012). Confirming our findings in animals with modifiable risk factors for stroke including increased age and hypertension is an important next step (Cho & Yang, 2018). Long-term survival studies are associated with mortality, and predetermined exclusion criteria were used to minimise type II errors. However, we recognise post-randomisation exclusion is itself a source of bias. This limitation could be mitigated by incorporating an 'intention to treat' analysis in future studies (Huang et al., 2020). Further, while a single endpoint for this longitudinal study reduced animal numbers, it is possible that the structural alterations responsible for enhanced performance were underestimated. The incorporation of non-invasive imaging modalities investigating duration of treatment could have provided improved temporal correlation between recovery and neuroanatomical changes and provided additional validation for the observed white matter changes.

In conclusion, our results show that citalopram provides medium-term functional recovery gains by modulating neuroinflammation and preserving the structural integrity of myelinated fibre tracts within the striatum in the subacute timeframe (days) post stroke. The extended time window for administration and the duration of functional benefits beyond the timeframe of administration suggest citalopram may be therapeutically useful alone or as an adjunct to physical rehabilitation in stroke patients who arrive late to hospital and are not suitable for thrombolytic therapy.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

SC was involved in study design, performed stroke surgeries, analysed results and drafted paper. LB provided input into experimental design and interpretation of results and revised the paper. ALM conceived and designed the study, analysed results and drafted and revised the paper.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ejn.15601.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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