

Research report

Social instability blocks functional restitution following motor cortex stroke in rats

Gergely Silasi^{a,*}, Derek A. Hamilton^{b,1}, Bryan Kolb^a

^a Department of Neuroscience, Canadian Centre for Behavioural Neuroscience, University of Lethbridge, Lethbridge, Alberta, Canada

^b Psychology, MSC03 2220, 1 University of New Mexico, Albuquerque, NM 87131-0001, USA

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Abstract

Social interactions have previously been shown to influence stroke outcome. In the current experiment we investigated the effects of a changing social environment on anatomical and behavioral recovery following motor cortex stroke in rats. Adult rats were trained on the Whishaw single pellet reaching task prior to receiving a devascularizing stroke lesion of the motor cortex. During the post-stroke testing period half of the rats were exposed to a form of social experience that has previously been shown to stimulate synaptic plasticity in frontal cortex circuitry, whereas the remaining rats were housed in pairs, in standard cages. At the end of the experiment the brains were processed for Golgi-Cox staining and dendritic length was measured in layer V of the intact forelimb motor area, layer III of Zilles' area Cg3 and layer II/III of Zilles' area AID. Social experience was found to completely block the normal spontaneous behavioural restitution in the lesion animals. Anatomically, whereas social experience selectively increased dendritic length in AID in rats that had not undergone behavioral training or the stroke procedure, this was not seen in the lesion animals, as the lesion alone produced an increase in dendritic length in both AID and Cg3. The findings are discussed in terms of the role of social experiences, including stress, on spontaneous plasticity that occurs following unilateral motor cortex stroke, and the effectiveness of inducing synaptic plasticity to promote behavioural recovery.

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1. Introduction

It is now well known that the post-injury environment has a significant effect on stroke outcome. For example, designated stroke units for clinical patients have been found to decrease the length of hospital stay, and also improve functional outcome relative to regular hospital care [1]. Animal studies have also shown that housing rats in an enriched environment after a stroke improves outcome in a number of different injury models [2–5]. Although the exact mechanisms of these beneficial effects are still largely unknown, it is believed that the combination of therapy and social interactions facilitates a general increase

in brain plasticity. Plastic processes in remaining brain regions have been found to play a major role in functional recovery following ischemic injury [6], therefore it is likely that environmental enrichment further promotes such plastic changes and thus improves functional outcome.

To further develop an understanding of the relationship between cortical plasticity and functional improvement following stroke, alternate methods of inducing the plastic changes should be investigated. In the current experiment we examined the effects of specifically engaging the orbital and medial prefrontal cortices of rats that received unilateral strokes of the motor cortex. To achieve this, rats were exposed to a form of social experience where the cage partners of the animals were rotated regularly within a group of previously familiarized rats. We have shown this form of social experience to be associated with plastic changes similar to those observed in somatosensory cortex following complex housing [7], but in this case the dendritic reorganization and synaptogenesis were only observed in the orbital and medial prefrontal cortices. Because prefrontal regions show spontaneous dendritic growth after motor cortex

* Corresponding author Present address: Centre for Neuroscience, Department of Psychology, P217 Biological Sciences Building, University of Alberta, Edmonton, Alberta T6G 2E9, Canada. Tel.: +1 780 492 5325/7142; fax: +1 780 492 1768.

E-mail addresses: silasi@ualberta.ca (G. Silasi), dahamilt@unm.edu (D.A. Hamilton).

¹ Tel.: +1 505 277 3060; fax: +1 505 277 1394.

injury [8], we hypothesized that our form of social experience would further enhance this process and thus facilitate functional recovery following motor cortex stroke.

2. Methods

2.1. Subjects and experimental procedures

Twenty-eight male, Long-Evans rats (90 days) that were born and housed in the vivarium at the University of Lethbridge were used for this study. The rats were maintained on a 12 h dark/light (19:00–07:00/07:00–19:00) cycle, and except for the food restriction period, food and water were available ad lib. During food restriction, each animal received only 30 g of food inside the home cage per day. Behavioural testing was carried out late in the afternoon and the social manipulation occurred after the behavioural testing, near the end of the light cycle. All procedures were in accordance with guidelines set by the Canadian Council on Animal Care and approved by the University of Lethbridge Animal Care Committees.

Twenty-four of the animals were divided into 4 weight-matched groups: stroke + no-treatment ($n=6$), stroke + social experience ($n=6$), control + social experience ($n=6$), control + no-treatment ($n=6$). Before initiating any experimental manipulations, the 6 animals in each group were housed together in a large cage (55 cm × 39 cm × 20 cm) for 18 days in order to become familiar with one another. Following this period of group-housing, single pellet reach training was initiated, and the animals were housed in pairs in standard-sized, hanging Plexiglas cages. The remaining animals ($n=4$) did not receive any behavioural training or surgical manipulations, but instead were used to quantify the dendritic effects of the social manipulation in untrained animals.

2.2. Single pellet reaching

Food-deprived animals were trained on the Whishaw single pellet reaching task as described by Whishaw [9]. The rats received daily reach training for 3 weeks before the lesion, and all groups had achieved a consistent level of accuracy. The reaching accuracy was calculated as the total number of successful reaches, divided by 25 (the total number of pellets presented) × 100. In order to aid in the scoring of the reaching behavior, test sessions were filmed on a ZR 30 MC Camcorder set at 1000th of a second shutter speed.

2.3. Devascularizing lesions of the motor cortex

Following the pre-training period, half of the animals received motor cortex devascularizing lesions [8] extending 4 mm anterior, 2 mm posterior and 4 mm lateral to bregma (1 mm lateral from the midline). Briefly, following craniotomy, the exposed dura was carefully removed and all of the underlying vasculature and pia was wiped away with a saline-soaked cotton swab. The incision was sutured shut and following the administration of an analgesic (2 mg/kg of Metacam; Boehringer Ingelheim) the animals were allowed to recover in separate cages on a heating blanket until they regained mobility. The following day, all animals were returned to the colony with their original cage partners.

2.4. Post-lesion manipulation and assessment

Following a day of recovery, the social experience was initiated in half of the animals and was continued for 5 weeks. For this procedure, animals in the social group received a new cage and bedding as well as a new cage partner every 48 h. Animals in the no-treatment groups only received a new cage and bedding. Although social animals received a different cage partner every 48 h the animals were generally familiar with one another as they were housed together for 18 days prior to reach training, and rats are known to be capable of maintaining social memories for many weeks [10]. In addition, the social switches were carried out only amongst the six animals in that particular group, therefore, every fifth switch resulted in the original cage partners being matched, from which point the original switching cycle was repeated. This rotation paradigm further facilitated the animals becoming more familiar with one another throughout the duration of the experiment.

Reach testing was initiated on the fourth post-surgical day. In order to minimize the effect of repeated testing on the rate of spontaneous recovery, the animals were tested for two consecutive days followed by 3 days with no reach testing. This pattern was repeated for 5 weeks following the lesion and each reaching session was video recorded. The group of animals that did not receive reach training was exposed to the social manipulation for the same length of time as the rest of the animals and was sacrificed thereafter.

2.5. Assessment of open field activity

We have previously investigated the effect of social experience on open field activity in uninjured animals [11], therefore in the current experiment we were interested in whether social experience altered the activity of animals with motor cortex lesions. Following the last day of single pellet reach testing rats were placed in a box (40 cm × 40 cm × 30 cm) equipped with a series of infrared sensors, which were connected to a Digiscan animal activity monitor (Omnitech, Columbus, OH, USA). The total horizontal and vertical activity over a 10-min testing period was summed for each animal [12], thus providing a single measure of total activity within the testing session.

2.6. Circulating corticosterone quantification

All animals were sacrificed on approximately post-surgical day 40 by administering an overdose of sodium pentobarbital. Once the rats were deeply anesthetized, a blood sample was collected from each rat and processed for serum corticosterone quantification using the Coat-A-Count radioimmunoassay (Diagnostic Products Corporation, LA, CA, USA). To ensure maximal accuracy of the results, each sample was run twice, and cases where the variability exceeded the pre-determined acceptable range for this assay were excluded from the analysis. Using these criteria, one sample (stroke + social) was excluded from the analysis.

2.7. Golgi-Cox dendritic analysis

To investigate the effects of unilateral motor cortex injury and social experience on dendritic morphology, the brains were processed for Golgi-Cox analysis [13]. Pyramidal cells from layer V of the forelimb area (FL), layer III of the anterior cingulate (Cg3) and layer II/III of the orbitofrontal (AID) cortices [14] were traced onto paper at 250X through a camera lucida drawing tube. In order for a cell to be considered for analysis it had to be: (I) well impregnated and unobstructed by other cells or blood vessels (II) both the apical and basilar trees had to be complete, with no broken or missing branches. The first five cells that fit the above criteria were drawn from each hemisphere. The cell drawings were then analyzed using the Sholl method [15], and the dendritic length of both the apical and basilar trees was calculated. Given that the animals received extensive unilateral skilled reach training, as well as unilateral cortical injury, initial analyses were carried out using hemisphere as a factor. In cases where there were no significant effects, the data were collapsed across hemispheres. The apical trees of cells in layer V of FL are usually truncated during histological preparation, and for this reason, only the basilar trees were analyzed from this area. To provide a consistent comparison across regions, the basilar dendritic length measures were also used for the analysis of cells in AID and Cg3, however the apical measures also showed similar trends (data not shown).

2.8. Lesion volume

Lesion volume was quantified at seven different planes in Golgi-Cox stained coronal sections of the brain. Digital images of the sections of interest were captured and the area of both the injured and uninjured hemispheres was measured at each plane of analysis using the NIH Image program (v1.62). The measurements were summed across planes and the difference between the remaining area of the injured and the uninjured hemispheres was calculated and multiplied by 100. The resulting value served as an indirect measure of infarct volume, as it expresses the area of remaining tissue in the injured hemisphere as a percentage of the contralateral, intact hemisphere.

3. Results

3.1. Single pellet reaching

By the end of pre-training all animals learned to reach for food in the reaching apparatus and the mean performance as a group was about 50%. Rats that received motor cortex strokes showed a significant drop in performance during the first post-operative testing session whereas the performance of the control groups did not change significantly. Animals in the stroke + no-treatment group showed a gradual increase in performance with subsequent testing sessions, although their performance on the last day, 5 weeks after the lesion, was still impaired relative to the control group. The stroke + social experience group had a similar initial impairment as the stroke + no-treatment group, but unexpectedly, they did not show the same spontaneous recovery with subsequent testing sessions. In fact, this group did not show significant improvement in performance at any point during post-surgical testing (Fig. 1a).

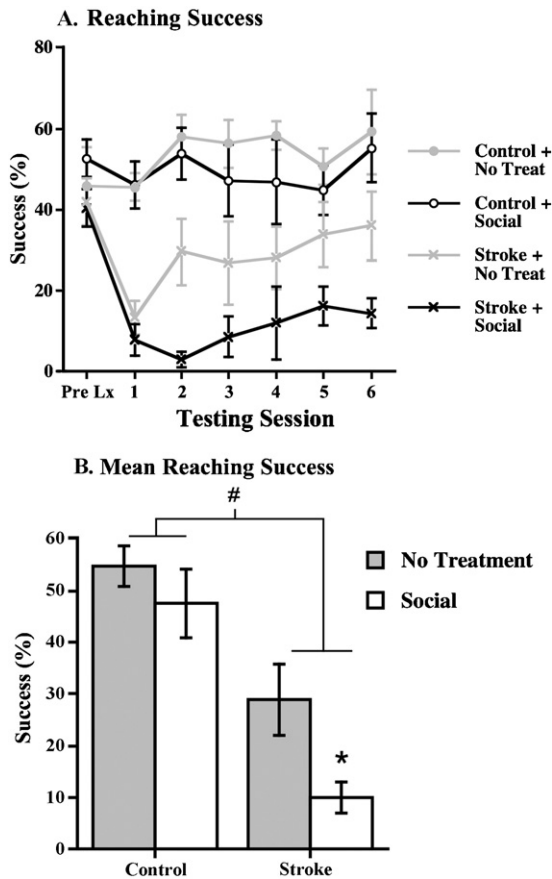


Fig. 1. Reaching performance in the Whishaw single pellet reaching task. Both stroke groups showed a significant impairment following the injury (A), and the stroke + no-treatment group showed significant improvement on subsequent testing sessions. The social experience completely blocked this spontaneous improvement, as there was no significant difference in reaching success between the first and the last post-surgical testing sessions. The mean post-stroke success rate (B) indicated that animals in the stroke + social group performed significantly worse than the stroke + no-treatment group (# indicates main effect of surgery, $P < 0.05$; * differs from stroke + no-treatment $P < 0.05$).

A two-way, repeated-measures ANOVA (surgery \times treatment) for mean post-surgical reaching success indicated that there was a main effect of surgery ($F(1, 19) = 32.452$, $P < 0.0001$), and of treatment ($F(1, 19) = 5.522$, $P = 0.0297$), but the interaction was not significant ($P = 0.307$). A Fisher's PLSD post-hoc analysis showed that the stroke + social group performed significantly worse, relative to the stroke + no-treatment group ($P = 0.0235$; Fig. 1b). An analysis of the mean number of post-surgical hits (successful reaches) in the stroke groups indicated that the stroke + social group had significantly fewer hits relative to the stroke + control group ($F(1, 10) = 6.239$, $P = 0.0316$), thus accounting for the lower success rate.

3.2. Open field activity

To determine if social experience influenced the activity of animals with unilateral motor cortex stroke, the activity of animals with stroke lesions was measured in an open field. There were no differences in total activity between the stroke + social and the stroke + no-treatment control group (Fig. 2a). A one-way ANOVA confirmed that treatment did not have a significant effect on activity in the open field ($F(1, 10) = 0.106$, $P = 0.7515$).

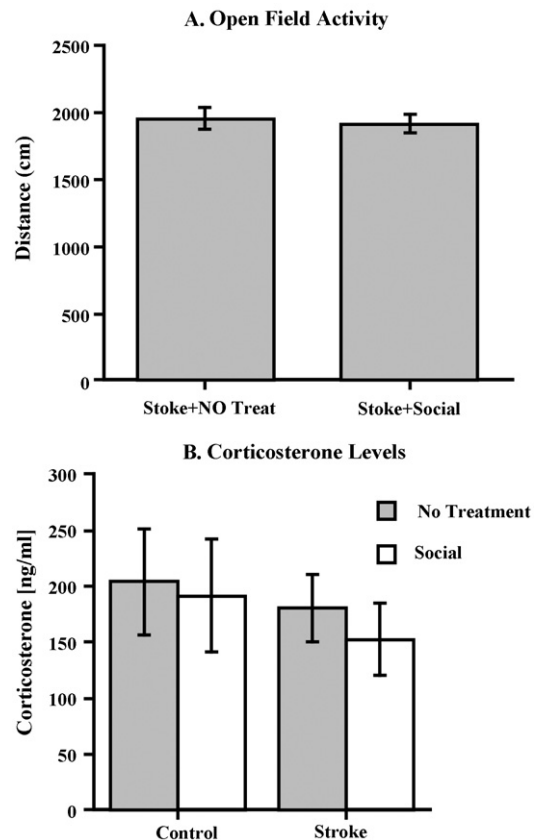


Fig. 2. (A) Total activity during 10 min of open field activity testing. Social experience did not alter the activity of animals with strokes. (B) Serum corticosterone levels at the end of the experiment as determined by the Coat-A-Count radioimmunoassay. Neither social experience nor the stroke had a significant effect on circulating corticosterone levels.

3.3. Serum corticosterone levels

A blood sample collected from each animal at the end of the experiment was used to quantify corticosterone levels. A two-way ANOVA (surgery \times treatment) demonstrated that neither the stroke ($F(1, 19)=0.552, P=0.4667$), nor the social manipulation ($F(1, 19)=0.233, P=0.6349$) had a significant effect on corticosterone levels (Fig. 2b). The surgery \times treatment interaction was also not significant ($P=0.8533$).

3.4. Infarct size

The stroke model used in the current experiment created an infarct that was mostly restricted to the cortex (Fig. 3a) and removed about 13% of the tissue in that hemisphere. Social experience did not have a significant effect on lesion volume (Fig. 3b), as a one-way ANOVA indicated that the area of the remaining hemisphere did not differ significantly between the social and the no-treatment groups ($F(1, 10)=0.023, P=0.8829$).

3.5. Dendritic morphology

Initial analyses were carried out to investigate differences in dendritic length between the injured and intact hemispheres,

however, there were no such differences in AID and Cg3, therefore the data were collapsed across hemispheres.

3.6. Orbital PFC (AID) in untrained rats

Animals that did not receive single pellet reach training showed significant dendritic changes in response to the social manipulation. As we have previously demonstrated in female animals [11], social experience in male rats also causes significant hypertrophy of the basilar dendrites of cells in AID (Fig. 4). A one-way ANOVA of basilar dendritic length indicated a main effect of group ($F(1, 4)=26.041, P=0.0070$).

3.7. Motor cortex (FL)

The stroke induced in the current experiment completely destroyed the FL area of the motor cortex on the side contralateral to the reaching paw, therefore no cells could be drawn from this area. In analyzing the length of the basilar dendrites of cells in the intact (non-reaching) hemisphere it was found that neither the injury itself nor the social experience produced any changes relative to control animals (Fig. 5a). A

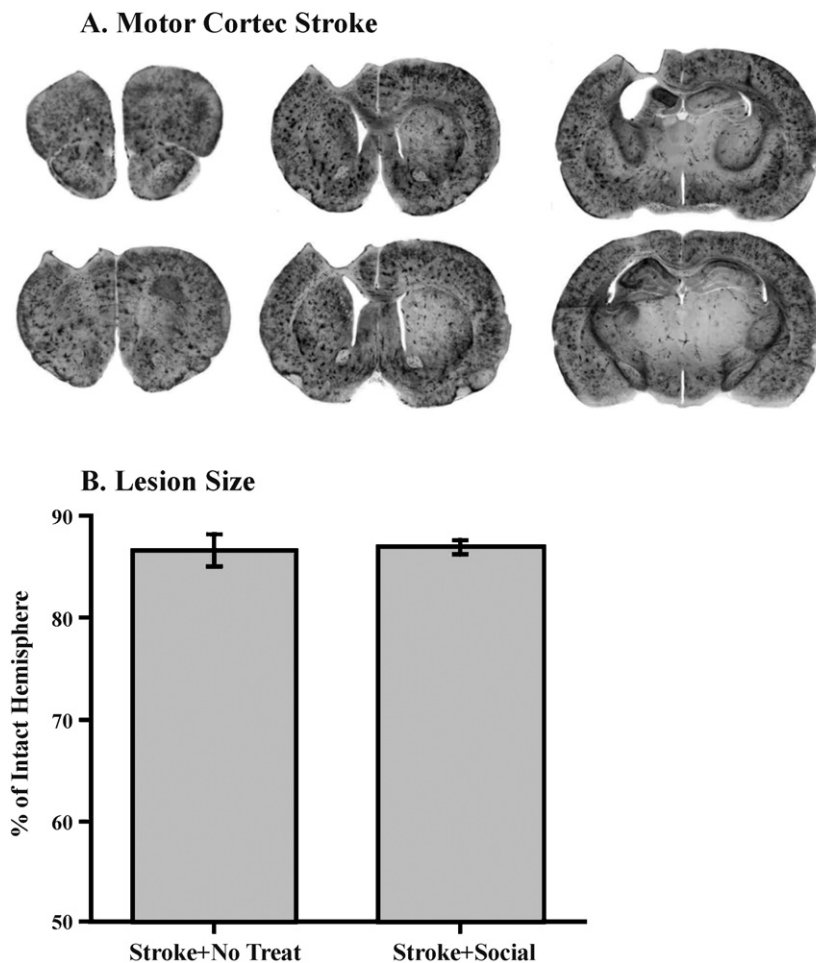


Fig. 3. Coronal sections showing the size and location of the devascularizing lesion. Damage was restricted mostly to primary motor cortex, however the lesion hemisphere often had enlarged ventricles and some damage to the corpus callosum (A). Lesion size did not differ between the stroke + no-treatment and the stroke + social groups (B).

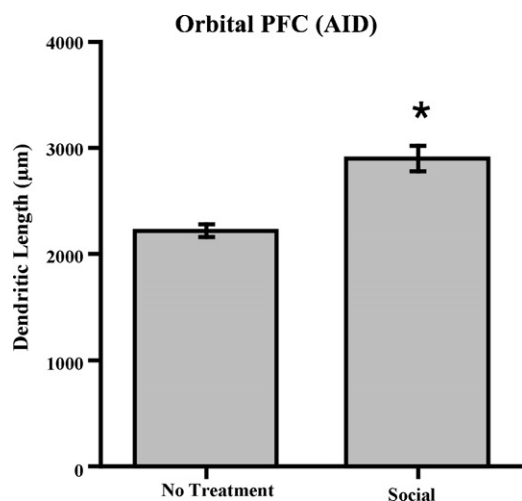


Fig. 4. Dendritic length of the basilar fields of layer II/III cells in the AID region of the prefrontal cortex in animals that did not receive reach training. Social experience was found to significantly increase dendritic length in the basilar field (* indicates $P < 0.05$).

two-way ANOVA (surgery \times treatment) indicated that there was no significant effect of lesion ($F(1, 18) = 0.515$, $P = 0.4820$), treatment ($F(1, 18) = 2.160$, $P = 0.1589$) nor the interaction ($P = 0.6942$).

3.8. Orbital PFC (AID) in trained rats

Animals that received strokes had significantly longer basilar dendrites relative to the un-operated group, but social experience did not have a significant effect on dendritic length (Fig. 5b). A two-way ANOVA (surgery \times treatment) of the basilar branches showed a significant surgery effect ($F(1, 41) = 9.515$, $P = 0.0036$), but no effect of treatment ($F(1, 41) = 0.331$, $P = 0.5680$), nor the interaction ($P = 0.3567$). To determine whether social experience in animals with stroke lesions had any significant effects at only the intermediate versus terminal portions of the dendritic arbor we performed a repeated measures ANOVA (using treatment as a factor) on the number of bisections at each of the Sholl rings. We found that the Sholl ring \times treatment interaction was not significant ($F(1, 15) = 3.175$, $P = 0.9994$), indicating that the treatment did not have differential effects along any of the dendritic segments.

3.9. Medial PFC (Cg3)

Compared to the un-operated group, animals that received strokes had a 15% increase in the length of the basilar dendrites, regardless of whether they received social experience or not (Fig. 5c). A two-way ANOVA (surgery \times treatment) for dendritic length of the basilar tree indicated a significant main effect of surgery ($F(1, 44) = 20.541$, $P < 0.0001$), but no treatment ($F(1, 44) = 0.068$, $P = 0.7961$), nor the interaction ($P = 0.4719$). To determine if there were any differential effects of the social experience along the dendritic segments we carried out a similar repeated measures ANOVA as above, and

found a non-significant ($F(1, 15) = 0.188$, $P = 0.9997$), Sholl ring \times treatment interaction.

4. Discussion

The results of the current experiment demonstrate that the spontaneous behavioural recovery that occurs after a unilateral motor cortex stroke can be completely blocked by exposing the animals to a form of social experience that presumably engages the frontal brain circuitry. In addition, we also show that following such stroke injury there are permanent structural changes in the orbital and medial prefrontal cortices.

The alteration of neuronal connections following cortical injury has been well documented in previous experiments, however most investigations have focused on quantifying these changes in remaining motor regions (e.g. [8,16]), and the exact causes of the dendritic changes are still poorly understood. One possible explanation for lesion-induced dendritic plasticity is that the behavioural adaptations following the injury are directly responsible for subsequent anatomical changes. For example, unilateral motor cortex stroke in rats causes dendritic hypertrophy in the intact motor cortex as well as an increase in the use of the non-affected forelimb [17]. Based on the strong temporal relationship between this behavioural and anatomical reorganization, it is believed that the behavioural compensation is most likely driving the structural changes in the intact hemisphere. In the current experiment we observed significant dendritic changes in two prefrontal cortical regions (outside of the motor cortex) and based on the known function of these areas we are able to draw certain conclusions about the nature of these changes.

The layer 2/3 cells in AID showed significant hypertrophy following the stroke. It is very unlikely though that these dendritic changes are directly related to the behavioural improvement that was observed following the injury, as this area does not have any motor projections to the spinal cord or other motor areas. A more likely explanation is that the hypertrophy in AID is driven by the increased reliance of the animals on olfactory input following the lesion-induced sensory-motor impairments. Given that rats spend a large portion of their time exploring their environment through tactile forepaw placements [18], the emergence of heightened sensory abilities in a different sensory domain might serve as an effective compensatory strategy following sensory-motor impairments. The orbital prefrontal cortex is known to subserve such olfactory function through extensive afferent projections that it receives from the olfactory bulb [19].

The medial frontal cortex (Cg3) also shows dendritic changes following motor cortex stroke and there are at least two arguments suggesting that the plastic changes in this region are actually facilitating the motor recovery. First, tracing studies have shown that projections arising from Cg3 that terminate in the striatum and the spinal cord subserve some motor function in the intact brain [20]. Furthermore, Whishaw et al. found that injury to the medial frontal cortex results in deficits during skilled reaching [21]. Given that the recovery following motor cortex injury is likely facilitated by plastic processes in remain-

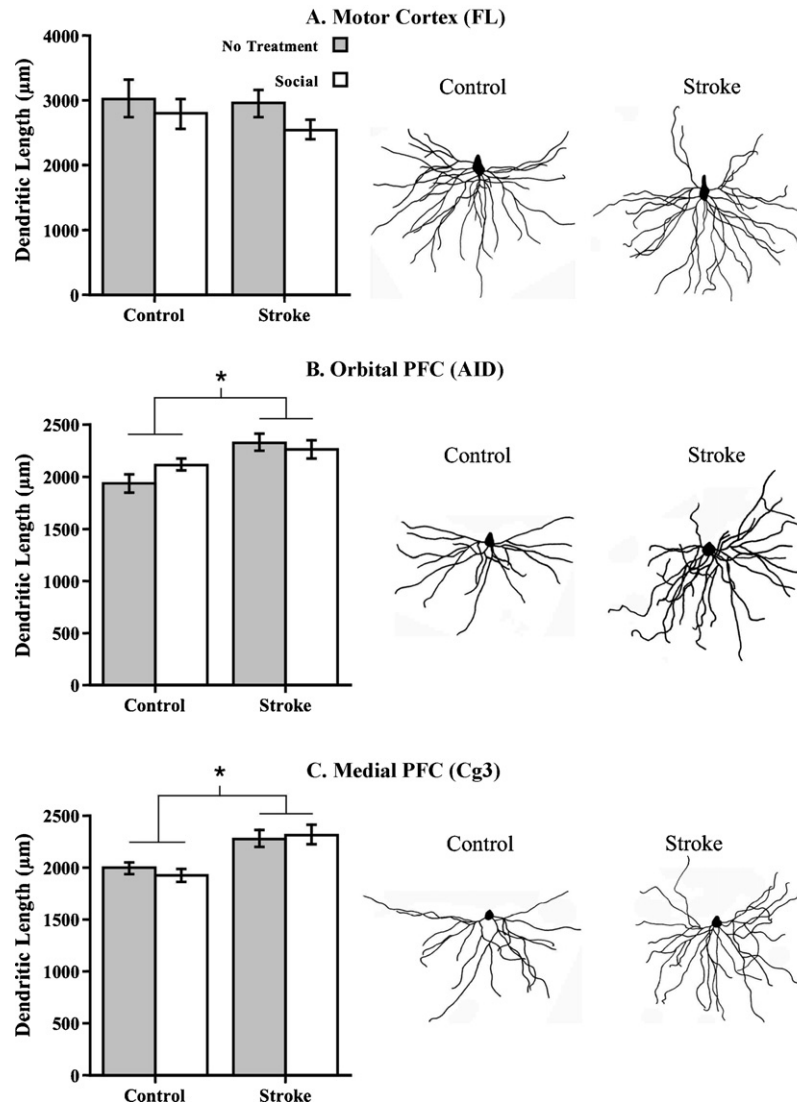


Fig. 5. (A) Dendritic length of the basilar fields of layer V neurons in the contralateral (to the stroke) motor cortex, (B) layer II/III cells in the orbital prefrontal cortex (AID), (C) and layer III cells in the medial prefrontal cortex (Cg3). Neither social experience nor the stroke injury had a significant effect on dendritic length in the forelimb region of the intact motor cortex (A). The stroke injury, however, caused a significant increase in dendritic length in both the orbital (B) and medial (C) prefrontal cortex. The cell drawings on the right are representative cells from the no treatment condition either with or without strokes (* indicates main effect of surgery, $P < 0.05$).

ing cortical regions, Cg3 would be the most likely region to overtake this function based on its existing motor connections. The second line of evidence comes from previous experiments that have reported dendritic reorganization of Cg3 neurons in conjunction with spontaneous [8], as well as treatment-induced recovery [22] following motor cortex lesions.

4.1. Effects of social experience

In previous experiments we have investigated the effects of the social experience paradigm used here on dendritic and synaptic reorganization in both the AID and Cg3 cortical regions of female Long-Evans rats [7]. We found that social experience increased dendritic length of neurons in AID, however Cg3 neurons showed no effect. In the current experiment we used male rats of the same strain and found that social experience induces

significant hypertrophy of AID cells in these animals as well. Surprisingly, however, when the social experience was administered in conjunction with behavioural training the dendritic changes were no longer significant. The behavioural training alone did not induce any dendritic changes in AID, suggesting that the region does not play a direct role in the acquisition of the motor skill. It is possible however, that the olfactory component of the reach training influenced the cells in AID in a way to block the subsequent effect of social experience. Specifically, the reach training may have altered the distribution of dendritic spines, which would likely alter the dendritic response of the cells to subsequent experience.

Even more surprising was the finding that the social experience completely blocked the behavioural recovery following the stroke lesion. We would anticipate that if the lesion-induced dendritic changes in the socially stable animals are somehow

related to the behavioural improvements, then the rats in the changing social environment ought to have improved functional outcome as well. It is possible that the dendritic length measurements are misleading here as they mask more subtle changes in synaptic reorganization. That is, the combination of injury- and experience-related synaptic reorganization may produce a different pattern of synaptic organization than the injury-related changes alone. The idea that synaptic growth might interfere with motor behavior has at least one precedent. Gonzalez et al. [23] have shown that modifying dendritic plasticity through the administration of nicotine also interferes with the learning of the same single pellet reaching task used here, and repeated doses of nicotine produce dendritic changes in both AID and Cg3 (e.g., [24]).

An alternate explanation of the behavioural results is that the poor behavioural recovery following the injury resulted from the effect of stress that may be associated with the social instability. It has been shown that stress can inhibit the skilled motor performance of intact animals as well as animals with brain injury [25]. In our experiment we quantified stress through open field activity as well as through the analysis of circulating corticosterone levels at the end of the experiment. Although these measures indicated that the social experience did not induce a chronic stress response, these measures did not tell us anything about the acute levels of stress, especially immediately following the injury. Nonetheless, if the animals were experiencing acute stress as a result of the social experience, it would be predicted that their performance in the single pellet reaching task would initially decrease, and then with time return to normal levels as the experience became less stressful. This trend, however, was not observed in the current experiment. It is also possible that the acute stress of social instability may have altered regions that we did not investigate here, such as amygdala or hippocampus, and it is the changes in these regions that affected the motor behavior. Another possible explanation for our unexpected negative effect of social experience on functional recovery after stroke is that social experience somehow affects the production of neurotrophic factors that are important for functional recovery. The expression of factors such as fibroblast growth factor-2 (FGF-2) and brain-derived neurotrophic factor (BDNF) is influenced by various forms of experience [26,27]. It is not known, however, how social experience might influence neurotrophic factor production. If social experience (or possibly associated stress) acted to suppress such production then we might expect some interference with spontaneous recovery.

Along the lines of our current results, a previous study by Woodlee and Schallert [28] has shown that social isolation, or removal of the original cage-partner, also has detrimental effects on anatomical outcome after stroke. Although in our study we attribute the negative behavioural effects of our treatment to the continuous rotation of the cage-partners after the injury, it is also possible that it is in fact the loss of the original cage-partner that is the key detrimental event.

Finally, we would be remiss if we did not comment on the relevance of our results to the treatment of stroke patients. Hospitals are characterized by social instability. There are new physicians, nurses, orderlies and with the exception of private rooms, there is

often considerable turnover in roommates. We can only wonder how these social factors might influence recovery from stroke.

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