Synergistic effects of age and stress in a rodent model of stroke

Dawn L. Merrett, Scott W. Kirkland, and Gerlinde A. Metz*

Canadian Centre for Behavioural Neuroscience, University of Lethbridge, 4401 University Drive West, Lethbridge, Alberta, Canada T1K 3M4

Abstract

Ageing and stress represent critical influences on stroke risk and outcome. These variables are intricately linked, as ageing is frequently associated with gradual dysregulation of the hypothalamic–pituitary–adrenal axis. This study determined the effects of stress on motor function in aged rats, and explored possible interactions of age and stress on motor recovery following stroke in a rat model. Young adult (4 months) and aged (18 months) male Wistar rats were tested in skilled and non-skilled movement before and after focal ischemia in motor cortex. One group of each age received restraint stress starting seven days pre-lesion until three weeks post-lesion. Aged rats were less mobile and stress further diminished their overall exploratory activity. Aged rats were also less proficient in motor skill acquisition and slower to improve after lesion. Stress diminished post-lesion improvement and prevented recovery of endpoint measures. The larger functional loss in aged rats vs. young rats was accompanied by greater damage of cortical tissue and persistent elevations in corticosterone levels. The behavioural and physiological measures suggest limited ability of aged animals to adapt to chronic stress. These findings show that age or stress alone can modulate motor performance but may have greater influence by synergistically affecting stroke recovery.

Keywords

Adverse experience; Glucocorticoids; Skilled reaching; Movement; Motor cortex; Focal ischemic infarct

1. Introduction

Age and stress are two of the most critical influences on stroke risk and recovery. It has been shown that the individual's capacity to recover from stroke depends on age [1–6]. It is possible that the increased vulnerability to stroke in elderly is linked to the cumulative effects of adverse experiences, such as stress, during a life-time [7,8]. In addition, ageing may be associated with gradual dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis leading to elevated glucocorticoid levels in elderly [9–11]. Increased basal circulating stress hormone levels [12] may contribute to age-related functional decline [13–15].

Similar findings have been made in experimental studies showing greater reduction in motor activity [16] and diminished performance in skilled and unskilled motor tasks in aged rats

*Corresponding author. Tel.: +1 403 394 3992; fax: +1 403 329 2775. gerlinde.metz@uleth.ca (G.A. Metz).

[17,28]. Sustained high levels of glucocorticoids may compromise the ability to overcome ischemia-induced deficits both on the neuronal and functional level [18–22]. Complex physiological changes involving activity of the HPA axis may explain, at least in part, the large variability of stroke recovery among aged individuals.

The purpose of the present study was to determine the influence of stress on motor function and recovery following stroke in aged rats. Young (3 months) and old (18 months) rats were trained to reach for small food pellets for comprehensive assessment of skilled limb use. Interactions of age and stress were investigated by exposing groups of animals to daily restraint, a stressor resembling mild psychological distress. The results revealed that age has a prominent detrimental effect on stroke outcome that is further exaggerated by stress.

2. Materials and methods

2.1. Subjects

Twenty-nine male Wistar rats (Charles River Laboratories, Senneville, QC) were used. Fifteen rats were approximately 4 months of age (370–405 g at the beginning of the study) and fourteen animals were 18 months of age (710–975 g at the beginning of the study). The animals were maintained in pairs in hanging Plexiglas cages at the University of Lethbridge vivarium on a 12-h light/dark cycle (8:00–20:00 h). Animals were placed on a restricted diet to maintain approximately 95% of their free-feeding weight to encourage participation in the reaching task. Throughout the study, the animals received a defined food ration in their home cages to maintain their body weight. All procedures were performed in accordance with the guidelines set forth by the Canadian Council for Animal Care.

2.2. Experimental design

Animals were assessed in unskilled and skilled motor performance. The single pellet reaching task was chosen as a measure of skilled forelimb use and motor learning. Once the animals reached asymptote success rates in the reaching task, they were divided into four groups: young lesion only ($n = 7$), young stressed ($n = 8$), old lesion only ($n = 7$), and old stressed ($n = 7$). All groups were trained and tested in the reaching task for six days per week for the duration of the study, with the exception of days when blood samples were taken. Both the young and old stress groups were given restraint stress for 20 min each day. After one week of stress and skilled reaching testing, all rats were given a unilateral devascularization lesion of the motor cortex. After the lesion, the rats were given three more weeks of daily stress and single pellet testing. Exploratory activity levels were measured at baseline, after stress, and at the end of weeks 1 and 3 post-lesion. Blood samples to measure circulating levels of corticosterone (CORT) were taken at baseline, after initiation of stress, day 1 after lesion, and three weeks after lesion. Twenty-three days after the lesion, animals were sacrificed and the brains extracted for histological analysis.

2.3. Motor cortex lesion

Rats were anesthetized with isoflurane (4% induction and 3% maintenance in 30% oxygen). Focal cerebral ischemia was induced to affect the rostral and caudal fore-limb areas of the motor cortex contralateral to the rat's preferred reaching paw. The ischemic infarcts were

induced through unilateral devascularization as described by Whishaw [23]. A craniotomy was performed using a fine dental drill at the following coordinates: –1.0 mm to 4.0 mm anterior and –1.5 mm to 4.5 mm lateral to Bregma. This section of skull and dura was removed, and the underlying blood vessels were gently wiped away with a sterile cotton swab. The skin was then sutured and the rat was given Temgesic (0.05 ml s.c.; Schering-Plough, ON, Canada). Rats recovered in individual cages placed on a thermoregulation pad until being returned to their home cages.

2.4. Blood samples

Rats were anesthetized with isoflurane and approximately 0.6 ml of blood was taken from the tail vein with a butterfly needle flushed with Heparin (LEO Pharma Inc., Thornhill, ON). The blood samples were centrifuged at 5000 rpm for 10 min. The plasma was extracted and stored at –20 °C until analyzed by radioimmunoassay (Coat-a-Count; Diagnostic Products Corporation, Los Angeles, CA). Blood samples were taken at baseline, three days after the initiation of restraint stress (Acute Stress), three days post-lesion (post-LX + stress), and after four weeks of daily restraint stress (chronic stress). Samples were taken at the same time of day and 10 min after the termination of restraint.

2.5. Restraint stress

Stress groups were treated with 20 min of restraint each day for four weeks. Clear Plexiglas tubes (7.6 cm inner diameter for young and 10.2 cm for old rats) were used that restrained the rats in a standing position. The tubes were adjustable in length and contained holes at the front for ventilation. The rats were tested in the single pellet reaching task 10 min after the termination of restraint stress [19].

2.6. Exploratory activity measures

Activity measurements were taken with the VersaMax Activity Boxes (AccuScan Instruments, Inc.), which used infrared beams to track the path of an animal when exploring the environment (see Fig. 3A). Rats were tested in the activity boxes for 5 min per session. The system recorded five 60-s samples of the amount of time spent moving and the total distance traveled.

2.7. Single pellet reaching task

This task was used to assess skilled forelimb use in rats prior to and after motor cortex lesion and stress treatment (see Fig. 4A). Individual rats were placed into a clear Plexiglas box that had a 1.2 cm wide vertical slit in the middle of the front wall of the box [24]. Mounted to the front wall was a shelf on which small food pellets were placed (45 mg banana-flavoured Dustless Precision Pellets, Bioserv, Frenchtown, NJ). The rats were trained to reach through the slot to retrieve a single pellet. Once animals started to show limb preference, pellets were placed into the indentation contralateral to the preferred paw. Rats were presented with 20 pellets in each session.

Through shaping, the rats were trained to walk to the back of the box between presentations of pellets, and this sequence of movements culminating in a reach was referred to as a trip [25]. This ensured that the rats were physically reoriented to the tray after each reach. Skill

training was recorded in pre-lesion training sessions by counting the number of trips made in a 10 min training session.

Endpoint performance was recorded by measuring success rates. A successful reach was recorded if the rat obtained and ate the pellet on the first attempt. Reaches that required multiple attempts to grasp the pellet were excluded from success rate calculations. Changes in reaching success over time were also recorded. Reaching success rates were also rated relatively to success rates on day 1 of stress treatment. Based on their success rates, animals were subdivided into reachers whose success rates showed an increase, a decline, or steady reaching performance [26]. The category of same reaching success included animals that showed no change in reaching success during pre-lesion stress treatment. Increased success was rated if the average reaching success increased during stress treatment and declined if the average reaching success dropped.

2.8. Histology

Animals were deeply anesthetized with an intraperitoneal overdose of Euthansol (Schering Canada Inc., Pointe-Claire, Quebec, Canada) and perfused through the heart with 0.9% saline followed by 4% PFA. Brains were extracted and placed in 4% PFA solution then transferred to a 30% sucrose solution.

Frozen coronal sections were cut at a thickness of 40- μ m, divided into ten series, and stained with cresyl violet to calculate infarct volume (see Fig. 1). Digital photographs of cresyl violet stained sections from the following ten planes (from Bregma) were analyzed: 3.70 mm, 3.20 mm, 2.70 mm, 2.20 mm, 1.70 mm, 1.20 mm, 0.70 mm, 0.20 mm, -0.30 mm, and -0.80 mm. The cross-sectional volumes of both hemispheres were calculated using a Zeiss Axiovision 4.3 microscope (Zeiss, Jena, Germany). The volume of the lesion was estimated using the Cavalieri method [27], and was expressed in terms of volume of the lesioned hemisphere as a percent of the volume of the contralateral intact hemisphere. Tissue distortion was evaluated using the following score: a score of 1 was given for minimal tissue displacement and a small enlargement of the ventricle; a score of 2 was given for moderate tissue displacement; a score of 3 was given for a large amount of tissue displacement, including tissue protrusion through the craniotomy window.

2.9. Statistical analysis

Statistical analysis was performed using SPSS 11.5 for Windows (SPSS Inc., Chicago). The behavioural data were analyzed with repeated-measures ANOVAs and with post hoc LSD tests for pairwise comparisons. When data did not meet the sphericity assumption, the Greenhouse-Geiser correction factor was applied and those *P*-values reported. The original degrees of freedom were retained for all corrected ANOVAs. Lesion volume was analyzed with a univariate ANOVA. Changes in reaching success after stress and tissue distortion after lesion were analyzed using the Kruskal-Wallis test and follow-up Mann-Whitney tests. A *P*-value of 0.05 was chosen as the significance level. All data are shown as the mean $\pm$ *SEM*.

3. Results

3.1. Old age exaggerates tissue displacement

The mean amount of tissue lost was about 10%, and no animal lost more than 15% of the volume of the lesioned hemisphere when compared to the intact hemisphere. All animals had visible cortical damage, but some animals had additional damage in the striatum or through the corpus callosum extending to the ventricle. There was no significant difference in lesion volume between groups (young lesion and young stress rats had approximately 89% of remaining intact tissue, the old lesion group 93.2%, and the old stress group 92.2%).

There were differences in tissue distortion, however. Fig. 1A–C illustrates a representative section for each of the lesion scores. There was a significant difference between old and young rats in the distribution of lesion scores ($P < 0.01$; see Fig. 1D). Old stress and old control rats showed larger degrees of tissue distortion than young control animals ($Z = -2.57$, $P < 0.01$; $Z = -2.42$, $P < 0.05$, respectively). There was no correlation between lesion volumes or tissue distortion to behavioural deficits.

3.2. Restraint stress and age affect plasma CORT levels

A repeated-measures ANOVA revealed an effect of time ($F_{3,63} = 7.78$, $P < 0.001$) and an effect of treatment with restraint stress ($F_{1,21} = 10.00$, $P < 0.01$). There was also a significant Time $\times$ Stress interaction ($F_{3,63} = 6.08$, $P < 0.001$), a Time $\times$ Age interaction ($F_{3,63} = 4.85$, $P = 0.004$) and an Age $\times$ Stress interaction ($F_{1,21} = 19.00$, $P < 0.001$). Two animals from each the old lesion group and the old stress group were excluded from the analysis because their intra-assay coefficient of variance was too great. There was a moderate acute stress effect for the young stress group ($P < 0.05$), while the CORT levels of the old stress group were elevated compared to baseline ($P < 0.001$) and compared to the old lesion group ($P < 0.001$). After lesion and after chronic stress, the young stress animals returned to their baseline CORT levels as opposed to the old stress animals that showed persistently high CORT values ($P < 0.001$) (Fig. 2).

3.3. Age and stress decrease exploratory activity

In the time spent moving, there were significant effects of time ($F_{4,100} = 11.73$, $P < 0.001$), age ($F_{1,25} = 46.36$, $P < 0.001$), and stress ($F_{1,25} = 9.06$, $P < 0.01$), and a Time $\times$ Age interaction ($F_{4,100} = 2.79$, $P < 0.05$). For total distance traveled, again there was a significant time effect ($F_{4,100} = 14.89$, $P < 0.001$), an age effect ($F_{1,25} = 58.71$, $P < 0.001$), a stress effect ($F_{1,25} = 10.168$, $P = 0.01$) and a Time $\times$ Age interaction ($F_{4,100} = 4.42$, $P < 0.01$). The old rats spent less time moving and traveled less distance than the young rats at all time points (all P -values < 0.01 ; see Fig. 3B and C). At the acute stress time point, the old stressed animals moved for less time and for a shorter distance than their lesion only counterparts ($P < 0.01$), but these differences disappeared by week 3 after the lesion.

3.4. Age influences skilled movement performance under stressful conditions

Skilled reaching success revealed an effect of time ($F_{4,92} = 20.72$, $P < 0.001$) and age ($F_{1,23} = 4.46$, $P < 0.05$). One old rat and one young rat were excluded from this analysis because they failed to perform successful reaches after lesion. Changes in reaching success after the

initiation of acute restraint stress revealed that none of the old rats improved over time as opposed to young animals (Fig. 4B). There was a significant difference in the number of young animals that showed an increased, declined or steady average reaching success after acute stress compared to old animals ($P < 0.05$). Over 70% of the young stress animals showed a greater than 5% improvement in success scores. Furthermore, comparisons of performance three weeks after lesion with baseline revealed that the young rats were able to significantly improve their success rates. In contrast, old rats were not able to re-attain their baseline scores after the lesion (all P s < 0.05).

3.5. Age and stress affect skilled reaching acquisition and post-lesion recovery

Skill learning was recorded in pre-lesion training sessions by recording the number of trips made in a training session. There was a main effect of time ($F_{31,775} = 122.43$, $P < 0.001$), age ($F_{1,25} = 81.99$, $P < 0.001$) and group ($F_{1,25} = 36.59$, $P < 0.001$), and a Time $\times$ Age interaction ($F_{31,775} = 21.52$, $P < 0.001$). Old rats displayed a slower acquisition of the task than young rats (see Fig. 4C). Old stress rats had significantly lower scores from day 4 until day 21 of training, while old controls remained lower up to day 26 and on days 28 and 29. By day 15 of training, all of the young rats were able to complete the task (20 trips, with a trip defined as walking to the back of the reaching box, turning and coming back to the front, and making an attempt to reach for the pellet) in 10 min or less. In contrast, some of the old rats were not able to complete the task until day 32 of training. Several old rats did not make any reaching attempts during the first 15 days of training.

From the first training day after the lesion, young rats were able to perform 20 trips in less than 10 min with few exceptions, while old rats were significantly impaired in performing the task for many days. There was an effect of time ($F_{16,400} = 10.83$, $P < 0.001$), age ($F_{1,25} = 11.94$, $P < 0.001$) and group ($F_{1,25} = 3.80$, $P < 0.05$), and a Time $\times$ Age interaction ($F_{16,400} = 8.60$, $P < 0.001$). Post hoc comparisons showed significant age differences for the first eight days of post-lesion training. Notably, old stress animals performed better than old controls on days 1, 2 and 8 after lesion (see Fig. 4C).

4. Discussion

The objective of this study was to investigate the influence of stress on motor performance and recovery after focal ischemic lesion in aged rats. Age reduced exploratory activity levels, which were further diminished by stress. Age also slowed the acquisition of a skilled reaching task. As opposed to young rats, post-stroke recovery in old rats was characterized by reduced proficiency in the skilled reaching task, slower re-learning and reduced endpoint performance. Stress further delayed motor recovery after lesion. The larger functional impairment in old lesion rats was accompanied by greater damage of cortical tissue and lasting elevations in circulating corticosterone levels. The data indicate persistent activation of the HPA axis in response to chronic stress in old rats as opposed to physiological habituation in young rats.

In the present study, age had a profound effect on motor function in the motor tasks. At baseline, old rats showed lower motor activity levels and were slower in acquiring the reaching task than younger ones. Accordingly, the young rats were able to attain baseline

scores within a week after lesion, while endpoint measures in old rats were still significantly lower at three weeks after lesion. Significantly fewer trips and reaches were made by the old rats until the second week after lesion. This slow reacquisition of the reaching task may be due to differences in the rate of adoption of compensatory movement strategies. These findings are supported by a recent study showing that compensatory movements are recruited in order to maintain skilled reaching success in aged rats, and they become even more vital for facilitating post-lesion recovery in aged vs. young rats [28].

While the acquisition of compensatory strategies most likely represents an implicit motor sequence learning process occurring across repeated training sessions, previous [3,28,29] and the present findings suggest that learning and plasticity in aged rats have limitations. Aside from permanently reduced post-lesion reaching success in old rats, the number of trips needed to perform reaching movements indicates a permanent disruption of previously learned movement patterns. Declines in learning ability of motor skills and impaired consolidation with ageing [29–31] may compromise recovery processes after ischemic lesion. Others, however, attempted to separate the factors of motor memory and physical ability [32]. Churchill et al. [32] report that impairments in procedural memory for a motor task in older animals may be partially attributed to a decline in general gross motor ability.

The decline in trips and reaching attempts displayed by old rats early after lesion may also indicate the formation of learned non-use. Learned non-use of skilled reaching in rats was shown to develop as a response of declined success during the acute phase of post-lesion testing [25]. Daily changes in reaching proficiency during the first days after lesion indicates that, though all groups showed learned non-use, this response was greater in stressed old rats. This change may be linked to depressive-like symptoms that are further exaggerated by HPA axis activation in stressed animals [33].

The present study used a common paradigm to induce mild to moderate psychological stress [34]. Although animals may show gradual habituation to daily restraint in physiological measures, it nevertheless causes chronically diminished motor deficits [19,22,35]. Similar findings were made in the young animals that displayed a modest increase in circulating CORT levels after three days of restraint and return to baseline levels at chronic time points. A large CORT increase was found in the old animals, indicating persistent activation of the HPA axis in response to chronic stress. These observations are in line with findings demonstrating a delay in recovery from stress in aged animals in addition to lasting CORT elevations [36]. Moreover, habituation to the stressor proceeds more slowly in aged animals [37]. This phenomenon may be the result of hypersecretion of glucocorticoids in response to a stressor or of reduced sensitivity to glucocorticoid feedback inhibition in aged animals [37,38].

The finding that old animals show a greater and persistent response to stress suggests that HPA axis activity represents a critical factor determining stroke risk and recovery in advanced age. This notion is supported by observations that sustained high cortisol levels may downregulate chaperone expression and thus compromise disease resistance [8]. Aged rats with ischemic insult were shown to develop larger infarct volumes [39–41] or greater tissue distortion than young animals [28]. These observations might be related to increased

necrosis, damaged blood–brain barrier, and enhanced microglial activation that were reported for aged animals [39–42].

The direction of stress-induced brain damage and behavioural changes may be also influenced by other variables such as rat strain. Previous studies using Lewis [18] and Long-Evans rats [19] demonstrated stress-induced alterations in skilled and non-skilled motor function. Differences between these and the present study might be explained by the relatively low reactivity to stress exhibited by the Wistar strain [43,44]. Their hypo-reactivity to stress might make Wistar rats an ideal model for ageing research and it strengthens the impact of the present findings for stroke research. Thus, the present data emphasize the importance and clinical relevance of using aged animals to study stroke and neurodegenerative conditions.

Acknowledgments

The authors would like to thank Keri Colwell, Nafisa Jadavji and Rebecca Supina for assistance with the experiments. This research was supported by the Canadian Institutes of Health Research (GM), the Canadian Stroke Network (SK, GM), and the National Sciences and Engineering Research Council of Canada (DM, GM). GM is a Senior Scholar of the Alberta Heritage Foundation for Medical Research.

Abbreviations

CORT	corticosterone
Post-LX	post-lesion

References

1. Macciocchi SN, Diamond PT, Alves WM, Mertz T. Ischemic stroke: relation of age, lesion location, and initial neurologic deficit to functional outcome. *Arch Phys Med Rehabil.* 1998; 79:1255–7. [PubMed: 9779680]
2. Kolb B, Cioe J, Whishaw IQ. Is there an optimal age for recovery from motor cortex lesions? II. Behavioural and anatomical consequences of unilateral motor cortex lesions in perinatal, infant, and adult rats. *Restor Neurol Neurosci.* 2000; 17:61–70.
3. Brown AW, Marlowe KJ, Bjelke B. Age effect on motor recovery in a post-acute animal stroke model. *Neurobiol Aging.* 2003; 24:607–14. [PubMed: 12714118]
4. Yager JY, Wright S, Armstrong EA, Jahraus CM, Saucier DM. The influence of aging on recovery following ischemic brain damage. *Behav Brain Res.* 2006; 173:171–80. [PubMed: 16899307]
5. Llinas, RH., Earley, C. Risk factors and primary and secondary prevention of stroke. In: Wityk, RJ., Llinas, RH., editors. *Stroke.* 2007. p. 21–38.
6. Chen RL, Balami JS, Esiri MM, Chen LK, Buchan AM. Ischemic stroke in the elderly: an overview of evidence. *Nat Rev Neurol.* 2010 Apr 6. Epub ahead of print.
7. Popovi IM, Seri V, Demarin V. Mild cognitive impairment in symptomatic and asymptomatic cerebrovascular disease. *J Neurol Sci.* 2007; 257:185–93. [PubMed: 17328916]
8. Kroll J. Correlations of plasma cortisol levels, chaperone expression and mammalian longevity: a review of published data. *Biogerontology.* 2010 Epub ahead of print.
9. Harper AJ, Buster JE, Casson PR. Changes in adrenocortical function with aging and therapeutic implications. *Semin Reprod Endocrinol.* 1999; 17:327–38. [PubMed: 10851572]
10. Heuser I, Deuschle M, Weber A, Kniest A, Ziegler C, Weber B, et al. The role of mineralocorticoid receptors in the circadian activity of the human hypothalamus-pituitary-adrenal system: effect of age. *Neurobiol Aging.* 2000; 21:585–9. [PubMed: 10924776]

11. Van Cauter E, Leproult R, Plat L. Age-related changes in slow wave sleep and REM sleep and relationship with growth hormone and cortisol levels in healthy men. *JAMA*. 2000; 284:861–8. [PubMed: 10938176]
12. Ferrari E, Cravello L, Muzzoni B, Casarotti D, Paltro M, Solerte SB, et al. Age-related changes of the hypothalamic–pituitary–adrenal axis: pathophysiological correlates. *Eur J Endocrinol*. 2001; 144:319–29. [PubMed: 11275940]
13. Buchman AS, Boyle PA, Wilson RS, Bienias JL, Bennett DA. Physical activity and motor decline in older persons. *Muscle Nerve*. 2007; 35:354–62. [PubMed: 17143881]
14. Potvin AR, Syndulko K, Tourtellotte WW, Lemmon JA, Potvin JH. Human neurologic function and the aging process. *J Am Geriatr Soc*. 1980; 28:1–9. [PubMed: 7350209]
15. Snijders AH, van de Warrenburg BP, Giladi N, Bloem BR. Neurological gait disorders in elderly people: clinical approach and classification. *Lancet Neurol*. 2007; 6:63–74. [PubMed: 17166803]
16. Torras-Garcia M, Costa-Miserachs D, Coll-Andreu M, Portell-Cortés I. Decreased anxiety levels related to aging. *Exp Brain Res*. 2005; 164:177–84. [PubMed: 15856210]
17. Janicke B, Schultze G, Coper H. Motor performance achievements in rats of different ages. *Exp Gerontol*. 1983; 18:393–407. [PubMed: 6667730]
18. Metz GA, Schwab ME, Welzl H. The effects of acute and chronic stress on motor and sensory performance in male Lewis rats. *Physiol Behav*. 2001; 72:29–35. [PubMed: 11239978]
19. Metz GA, Jadavji NM, Smith LK. Modulation of motor function by stress: a novel concept of the effects of stress and corticosterone on behavior. *Eur J Neurosci*. 2005; 22:1190–200. [PubMed: 16176362]
20. Madrigal JL, Caso JR, de Cristobal J, Cardenas A, Leza JC, Lizasoain I, et al. Effect of subacute and chronic immobilisation stress on the outcome of permanent focal cerebral ischaemia in rats. *Brain Res*. 2003; 979:137–45. [PubMed: 12850580]
21. Kirkland SW, Coma AK, Colwell KL, Metz GA. Delayed recovery and exaggerated infarct size by post-lesion stress in a rat model of focal cerebral stroke. *Brain Res*. 2008; 1201:151–60. [PubMed: 18308294]
22. Zucchi FCR, Kirkland SW, Jadavji NM, van Waes LT, Klein A, Badr N, et al. Predictable stress versus unpredictable stress: a comparison in a rodent model of stroke. *Behav Brain Res*. 2009; 205:67–75. [PubMed: 19573561]
23. Whishaw IQ. Loss of the innate cortical engram for action patterns used in skilled reaching and the development of behavioral compensation following motor cortex lesions in the rat. *Neuropharmacology*. 2000; 39:788–805. [PubMed: 10699445]
24. Metz GA, Whishaw IQ. Skilled reaching an action pattern: stability in rat (*Rattus norvegicus*) grasping movements as a function of changing food pellet size. *Behav Brain Res*. 2000; 116:111–22. [PubMed: 11080542]
25. Erickson CA, Gharbawie OA, Whishaw IQ. Attempt-dependent decrease in skilled reaching characterizes the acute postsurgical period following a fore-limb motor cortex lesion: an experimental demonstration of learned nonuse in the rat. *Behav Brain Res*. 2007; 179:208–18. [PubMed: 17346809]
26. Metz GA, Antonow-Schlorke I, Witte OW. Motor improvements after focal cortical ischemia in adult rats are mediated by compensatory mechanisms. *Behav Brain Res*. 2005; 162:71–82. [PubMed: 15922067]
27. Schmitz C, Hof PR. Design-based stereology in neuroscience. *Neuroscience*. 2005; 130:813–31. [PubMed: 15652981]
28. Alaverdashvili M, Whishaw IQ. Compensation aids skilled reaching in aging and in recovery from forelimb motor cortex stroke in the rat. *Neuroscience*. 2010; 167:21–30. [PubMed: 20149844]
29. Flöel A, Breitenstein C, Hummel F, Celnik P, Gingert C, Sawaki L, et al. Dopaminergic influences on formation of a motor memory. *Ann Neurol*. 2005; 58:121–30. [PubMed: 15984008]
30. Bickford P, Heron C, Young DA, Gerhardt GA, De La Garza R. Impaired acquisition of novel locomotor tasks in aged and norepinephrine-depleted F344 rats. *Neurobiol Aging*. 1992; 13:475–81. [PubMed: 1508298]

31. Vakil E, Agmon-Ashkenazi D. Baseline performance and learning rate of procedural and declarative memory tasks: younger versus older adults. *J Gerontol B: Psychol Sci Soc Sci.* 1997; 52:P229–234. [PubMed: 9310091]
32. Churchill JD, Stanis JJ, Press C, Kushelev M, Greenough WT. Is procedural memory relatively spared from age effects? *Neurobiol Aging.* 2003; 24:883–92. [PubMed: 12927770]
33. Fuchs E, Flügge G. Psychosocial stress induces molecular and structural alterations in the brain—how animal experiments help to understand pathomechanisms of depressive illnesses. *Z Psychosom Med Psychother.* 2001; 47:80–97. [PubMed: 11593455]
34. Flores C, Hernandez M, Hargreaves K, Bayer B. Restraint-stress induced elevations in plasma corticosterone and beta-endorphin are not accompanied by alterations in immune function. *J Neuroimmunol.* 1990; 28:219–25. [PubMed: 2142692]
35. Smith LK, Jadavji NM, Colwell KL, Pehudoff KS, Metz GA. Stress accelerates neural degeneration and exaggerates motor symptoms in a rat model of Parkinson's disease. *Eur J Neurosci.* 2008; 27:2133–46. [PubMed: 18412632]
36. Sapolsky RM, Krey LC, McEwen BS. The adrenocortical stress-response in the aged male rat: impairment of recovery from stress. *Exp Gerontol.* 1983; 18:55–64. [PubMed: 6683660]
37. Sapolsky RM, Krey LC, McEwen BS. The adrenocortical axis in the aged rat: impaired sensitivity to both fast and delayed feedback inhibition. *Neurobiol Aging.* 1986; 7:331–5. [PubMed: 3024042]
38. Sapolsky, RM. *Stress, the aging brain, and the mechanisms of neuron death.* Cambridge, MA: MIT Press; 1992.
39. Sutherland GR, Dix GA, Auer RN. Effect of age in rodent models of focal and forebrain ischemia. *Stroke.* 1996; 27:1663–8. [PubMed: 8784145]
40. Gong Y, Hua Y, Keep RF, Hoff JT, Xi G. Intracerebral hemorrhage: effects of aging on brain edema and neurological deficits. *Stroke.* 2004; 35:2571–5. [PubMed: 15472083]
41. DiNapoli VA, Huber JD, Houser K, Li X, Rosen CL. Early disruptions of the blood–brain barrier may contribute to exacerbated neuronal damage and prolonged functional recovery following stroke in aged rats. *Neurobiol Aging.* 2008; 29:753–64. [PubMed: 17241702]
42. Badan I, Buchhold B, Hamm A, Gratz M, Walker LC, Platt D, et al. Accelerated glial reactivity to stroke in aged rats correlates with reduced functional recovery. *J Cereb Blood Flow Metab.* 2003; 23:845–54. [PubMed: 12843788]
43. Henry JP, Liu YY, Nadra WE, Qian CG, Mormede P, Lemaire V, et al. Psychosocial stress can induce chronic hypertension in normotensive strains of rats. *Hypertension.* 1993; 21:714–23. [PubMed: 8491506]
44. Tohei A, Mogi Y, Kon H, Hokao R, Shinoda M. Strain difference in pituitary-adrenal axis between Wistar–Imamichi and Long Evans adult male rats. *Exp Anim.* 2003; 52:437–9. [PubMed: 14625413]

Fig. 1.

Tissue damage produced by the motor cortex lesion. Photomicrographs of cross-sections showing (A) large lesion with no tissue displacement (score of 1); (B) moderate tissue displacement, damage to the ventricle and corpus callosum (score of 2); (C) substantial tissue displacement (score of 3). (D) Distribution of lesion scores. Note that old control and old stress animals had higher average scores than young animals. Staining: cresyl violet; scale bar: 1.0 mm. Asterisks indicate significances: ** $P < 0.01$, difference between old groups and young control animals.

Fig. 2.

Plasma corticosterone (CORT) levels at baseline, after three days of restraint stress, three days after lesion, and after four weeks of stress. Note that young and old animals responded differently to stress, with older animals exhibiting persistently elevated CORT levels throughout four weeks of chronic restraint stress. Asterisks indicate significances: *** $P < 0.001$ differences between lesion only and stress groups; # $P < 0.05$, ### $P < 0.001$ differences from baseline within each group. Post-LX: post-lesion.

Fig. 3.

Exploratory activity at baseline, after initiation of restraint stress, and one and three weeks after motor cortex lesion. (A) Time spent moving (s); (B) total distance traveled (cm). Note that old age and acute stress decreased exploratory activity levels. Asterisks indicate significances: *** $P < 0.001$, difference between young and old groups; # $P < 0.05$, ## $P < 0.01$, difference between lesion only and stress groups; † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$, difference from baseline within each group.

Fig. 4. Skilled reaching performance. (A) Photograph illustrating the single pellet reaching task. (B) The number of young and old animals that showed an increased, a declined or maintained reaching success after acute stress. (C) The acquisition (initial training) and reacquisition (after motor cortex lesion) of the single pellet reaching task by young and old rats. Note that none of the old rats improved in reaching success after stress initiation. Old rats were also slower to learn the task and were impaired at completing the task for one week after motor cortex lesion. Asterisks indicate significances: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, differences between old groups and young control animals; # $P < 0.05$, ## $P < 0.01$, difference between lesion only and stress groups.