Sex differences in skilled movement in response to restraint stress and recovery from stress

Nafisa M. Jadavji and Gerlinde A. Metz*

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

Abstract

Sex differences exist in skilled movement, and skilled motor performance is also influenced by stress. As shown for cognitive function, the effects of stress are usually characterized by considerable sexual dimorphism. The purpose of this study was to investigate sex differences in skilled motor function in response to stress. Male and female Long–Evans rats were trained and tested in skilled reaching and skilled walking tasks. Both groups of animals were then exposed to daily restraint stress for 15 days. Recovery from daily stress was assessed by comparing reaching performance at 10 min versus 60 min after restraint stress, and recovery from chronic stress was tested for 21 days after cessation of stress. Animals were tested daily in skilled reaching for the entire period. Observations showed that females performed significantly better than males during the stress period in terms of reaching success and number of attempts needed to grasp a food pellet. No difference between testing at 10 or 60 min after daily stress was found. Analysis of movement patterns and recovery from stress indicated that males and females use different strategies to overcome stress-induced motor disturbance. While male rats preferred to use original movement patterns, females tended to modify these patterns in order to increase reaching success. Modification of movement patterns in female rats was accompanied by a faster recovery in success rate after the cessation of stress. These results indicate sex differences in skilled reaching in response to stress, and in the recovery period after stress.

Keywords

Male; Female; Skilled movement; Skilled reaching; Motor function; Recovery

1. Introduction

The behaviour of males and females can differ considerably. For example, sex differences have been found in motor performance, including limb and body movements [1]. Previous studies have shown sex differences in hind limb use during vertical exploration [2] and sexually dimorphic postural adjustments in preparation for skilled reaching [3]. It has been hypothesized that these different postural strategies are related to differential exposure to gonadal hormones during perinatal development [4].

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

In addition to behavioural performance, males and females also show differences in their response to experience. For example, female rats seem more resistant to stress than males in cognitive performance [5–8]. The sexually dimorphic behaviour during stress has been related to fundamental physiological differences between males and females. For example, a study in humans found that males secrete higher levels of adrenaline when compared to females of the same age while exposed to identical stressors [9]. Sex differences in response to stress in rats have also been identified [10,11]. Carey et al. [12] reported that activity of the hypothalamic–pituitary–adrenal (HPA) axis in female rats depends on the stage during estrous cycle. These physiological differences may result in differential responses towards presentation of a stressor. For instance, exposure to repeated immobilization stress leads to decreases in body weight in male but not female rats [13].

Sexual dimorphism in the physiological status might also determine behavioural responses to stress, including learning and memory, and motor performance. In general, male rats seem more prone to stress-induced disturbance of memory function [14]. The investigation of stress-induced sex differences in motor function so far was limited to assessment of open field locomotion. Acute stress causes a decrease in locomotor activity in an open field in males, while females seem to be less sensitive [10,13]. In addition, locomotor effects of stress depend on rat strain with Long–Evans rats being less sensitive than other strains [13]. Recent studies have also shown that stress is a potent influence on both skilled and non-skilled movement performance [15–17]. With regard to sex differences in both motor performance and the stress response, it is reasonable to expect that gender and stress represent interacting factors to affect motor system function.

The purpose of the present study was to explore possible sex differences in motor performance in response to stress in rats. The rats' performance was tested in skilled reaching and skilled walking tasks, which represent highly sensitive tests to display slight aberrations in limb use and bodily adjustments [2,3,16]. Recovery from daily stress was assessed by comparing reaching performance at 10 min versus 60 min after restraint stress, and recovery from chronic stress was tested after cessation of the chronic stress period. The observations revealed a moderate influence of acute and chronic stress on quantitative and qualitative aspects of reaching in male and female rats. Most interestingly, the largest sex differences were found in the course of recovery after cessation of stress.

2. Materials and methods

2.1. Subjects

Subjects were 10 male and 10 female adult Long–Evans hooded rats, approximately 4 months old and raised at the University of Lethbridge vivarium. Males weighed between 400 and 600 g and females between 250 and 350 g at the beginning of the experiment. Animals were housed in groups of two or three in Macrolon cages (43 cm × 20.4 cm × 25.7 cm) under a 12:12 h light/dark cycle with light starting at 07:30 h. The housing room was maintained at a temperature of 20 °C and 30% relative humidity. One male rat died during the experiment and its behavioural data was included in all baseline and chronic stress statistics, but was not included in the post-stress interval statistics.

Prior to the experiment, rats were food deprived to 95% of their initial body weight to encourage participation in the reaching task. To maintain body weight, supplementary food was given daily in the home cage 5 h after behavioural testing. Once animals had acquired the reaching task, they were allowed to gain weight, which did not compromise their continued participation in this task. Animals were weighed daily throughout the experiment. Water was available *ad libitum*. The experiment was performed according to the standards set by the Canadian Council of Animal Care as approved by the University of Lethbridge Animal Welfare Committee.

2.2. Experimental design

Testing and training of the animals was performed during the light phase of the cycle each day at the same time in the morning hours. Fig. 1 illustrates the time course of manipulations and behavioural measurements. Rats were initially trained in the skilled reaching and ladder rung walking tasks. Twenty-one days later, baseline measurements for the skilled reaching task were taken from the last 5 days of training. Skilled reaching, ladder rung walking and open field were video recorded on the following day (Baseline). Baseline blood samples to analyze circulating corticosterone levels were collected the day after behavioural baseline measures were completed and 1 day before stress commenced. All animals were exposed to 15 days of daily restraint stress and tested daily in the skilled reaching task for 14 days of this period. Stress procedures and behavioural testing occurred at the same time in the morning hours each day. To compare for effects of the post-stress interval after daily restraint, half of the animals were tested 10 min after stress and the other half, 60 min after stress. Chronic stress video recordings of reaching and ladder rung walking were taken and open field activity was measured on day 14 of stress (Chronic Stress). Blood samples were collected on day 15 of stress. After cessation of the stress period, all animals continued to be tested daily in skilled reaching until stable asymptotic reaching success rates were observed (day 21 post-stress). At the end of this interval animals were again video recorded in skilled reaching, ladder rung walking and open field tasks. Behavioural post-stress testing was terminated for all animals at 21 days. Blood samples were again collected at day 22 post-stress (post-stress). At the time of each blood collection (baseline, chronic stress, and post-stress), blood glucose concentrations were also measured to obtain an additional indicator of stress severity.

2.3. Restraint stress

Animals were placed individually in custom-made transparent Plexiglas containers (5 cm inner diameter) for a period of 20 min each day [13,18,19]. The containers had perforated ends to allow for ventilation. The containers maintained the animals in a standing position with slight compression of the body.

3. Behavioural testing and analysis

3.1. Skilled reaching task

3.1.1. Skilled reaching task apparatus—The reaching boxes were made of clear Plexiglas (40 cm × 45 cm and 13.1 cm wide). Animals extended their forelimbs to reach for food pellets through a 1.3 cm wide vertical opening in the middle of the front wall (Fig. 2A).

The vertical opening extended from the floor to a height of 15 cm. To hold the food pellet, a 2 cm wide by 4 cm long shelf was positioned outside the front wall of the box. The shelf was mounted 4 cm above the floor. Food pellets (45 mg banana flavored Dustless Precision Pellets, BioServ Inc., Frenchtown, NJ) were placed in one of two small indentions on the shelf. The indentations, each 5 mm in diameter and 1.5 mm deep, were 2 cm away from the inside wall of the box and were centered on the edges of the slit through which the rats reached [20].

3.1.2. Training and testing—Once rats began to reach for food, food was placed in the indentation contralateral to the limb that the rat used for reaching. Between individual reaching movements, rats were required to leave the food aperture and walk to the rear end of the box in order to reposition themselves prior to the next reach. Each training and testing session required the rats to reach for 20 food pellets. Reaching performance was scored by counting misses and successful reaches for each limb [20]. An ‘attempt’ was defined as a repeated forelimb movement towards the pellet and obtaining the pellet after more than one reach. A ‘success’ was recorded if an animal grasped a food pellet on the first attempt and withdrew the paw with the pellet through the slit to consume the food. A ‘miss’ was recorded if an animal touched and missed the pellet using more than one attempt to grasp it [21]. Percent reaching success was calculated by counting the number of successful reaches divided by the number of pellets given in each session (20) multiplied by 100.

3.1.3. Video taping and analysis—On the last day of baseline, stress, and post-stress test sessions, the animals’ performance was video recorded from a frontal view for qualitative movement analysis. The rating of the reaching movements was performed from the videotapes by frame-by-frame inspection. The first three successful reaches were scored. Movements were analyzed using a framework derived from the Eshkol–Wachman movement notation which allows analysis of the relations and changes of relation between parts of the body and limbs [22]. The following movement components of reaching were analyzed from a frontal view [20,23,24]: (1) *Orient*, (2) *Limb lift*, (3) *Digits close*, (4) *Aim*, (5) *Advance*, (6) *Digits open*, (7) *Pronation*, (8) *Grasp*, (9) *Supination I*, (10) *Supination II*, and (11) *Release*. To enhance the resolution for correlation analysis, each of the individual subcomponents was also rated using a previously described 35-point scale [20].

For each of the 11 movement components, a score of 0 was given when the movement was absent, a score of 0.5 was given if the movement was present but abnormal, and a score of 1 was given if the movement was normal [20].

3.2. Skilled walking task

3.2.1. Ladder rung walking task apparatus—The horizontal ladder was made of two side-walls (1 m long and 20 cm high) of clear Plexiglas with metal rungs (3 mm in diameter), inserted at random distances ranging from 1 to 5 cm to create a floor (Fig. 4A [25]). The irregular pattern was used to maintain the difficulty of the task across repeated test sessions. The ladder was elevated 30 cm above ground with a neutral start box and the animals’ home cage at the end.

3.2.2. Training and testing—A training session included five trials. Each trial required the animal to cross the length of the ladder to reach the home cage placed at the end of the apparatus. One training session was administered, with the baseline test session on the following day. One test session was performed at baseline, pre-lesion and post-lesion time points. Each test session consisted of three trials during which the animals' performance was videotaped.

3.2.3. Video taping and analysis—Ladder rung walking performance was video-recorded from a lateral perspective [25]. The camera was positioned at a slight ventral angle, so that both sides and the paw positions could be recorded simultaneously from a ventral view. The tapes were analyzed frame-by-frame for quantitative and qualitative analysis. Quantitative analysis was based on the number of errors in each crossing. Based on the limb placement scoring system (see below), an error was defined as a limb placement that involved missing the rung or slipping off the rung (score of 0, 1 or 2 points according to the scale). The mean number of errors per step of each fore- and hind limb was calculated and averaged for three trials. The errors for the contralateral fore- and hind limbs were averaged and expressed as percent errors per total number of steps.

The qualitative analysis of forelimb and hind limb placements was performed using the foot fault scoring system developed earlier [25]. Consecutive steps were analyzed, excluding the last step before a pause and the first step after a pause. The last stepping cycle at the end of the ladder rung apparatus was also excluded from scoring. Limb placement was scored by categorizing the placement of the limb on a rung and the limb protrusion between rungs when a miss occurred by using a 7-category scale [25]: (1) *Total miss*; (2) *Deep slip*; (3) *Slight slip*; (4) *Replacement*; (5) *Correction*; (6) *Partial placement*; (7) *Correct placement*.

An error was counted when an animal missed a rung or slipped off (error scores of 0, 1 and 2). Percent error was calculated by dividing the number of errors by total number of steps. The number of foot placement errors was calculated as a percentage of the total number of steps made in a respective trial.

3.3. Open field task

3.3.1. Open field apparatus—The open field box, measuring 100 cm × 100 cm × 18 cm, was made of opaque black Plexiglas. The bottom of the box was divided into 16 zones (22 cm × 22 cm) using white masking tape (Fig. 5A).

3.3.2. Testing—Each rat was individually placed in the middle of the open field box and video recorded for 5 min [26]. After testing of each rat was completed, the floor of the box was cleaned with soap.

3.3.3. Video taping and analysis—Video recordings were scored for vertical activity, horizontal activity (total number of fields entered), and % time spent in the center and outside fields. Entered fields were scored when more than 50% of the animal's body crossed a subdivision of the open field.

3.4. Video recording procedures

Videotaping in all tasks was performed using a Sony ZR70 portable digital video camera. The shutter speed was set at 1/500 s. Tapes were analyzed frame-by-frame on a Sony Mini DV player. The testing setup was illuminated by a white light source. In addition, the skilled reaching apparatus was also illuminated by a one-arm cold light source (Schott, Jena, Germany).

3.5. Corticosterone, glucose and body weight measurements

Blood samples were collected 10 min after restraint stress between the hours of 09:00 and 10:30. Rats were anesthetized using 4% isoflurane. Anesthesia lasted less than 5 min in which 1.0 ml of blood was collected from the tail vein. Syringes and needles used to collect blood were rinsed with the anticoagulant heparin. Blood glucose was measured using an Ascensia Breeze Blood Glucose Meter (Bayer, Toronto, ON). The remaining blood was transferred to centrifuge tubes and plasma was obtained by centrifugation at 10,000 rpm for 8 min. The samples were stored at -20°C . Plasma corticosterone levels for baseline and chronic stress time points were determined by a radioimmunoassay (RIA) kit for corticosterone (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA [27]) and for post-stress by enzyme linked immunosorbent assay (ELISA [28]). ELISA analyses were performed using corticosterone EIA Kits (Cayman Chemical, Ann Arbor, MI). Limits of detection were between 20 and 2000 ng/ml for RIA and 24–10,000 pg/ml for ELISA analysis. Corticosterone concentrations with a coefficient of variation, less than 15% for the RIA and 20% for the ELISA were used for statistical analysis. Both RIA and ELISA methods have previously been shown to produce similar results for progesterone in dairy milk [29].

For further evaluation of stress-induced physiological changes, body weight of the animals was recorded every day. To account for the different weight among males and females, the data were analyzed as percentage of baseline values.

3.6. Statistical analysis

Statistical analysis was performed using a SPSS software package 11.5 (SPSS Inc., Chicago, IL, 2002). The results were subject to one-way and two-way analyses of variance (ANOVA) for repeated measures across testing sessions. These data were further investigated by comparing means and variances between groups using unpaired *t*-tests, and paired *t*-tests for within-subject comparison. When no significant difference between animals tested 10 and 60 min after exposure to restraint stress was found, the data reported are based on pooled data of both groups. In all statistical analyses, a *p*-value of less than or equal to 0.05 was considered significant. All data are presented as mean $\pm$ standard error of the mean.

4. Results

4.1. Skilled reaching

4.1.1. Reaching success—During baseline tests both males and females obtained 10 out of 20 pellets successfully, resulting in a $55.61 \pm 1.61\%$ success rate (Fig. 2B). There was no significant difference in success rates between males and females at this time. Both males

and females had significantly lower reaching success on the first day of stress when compared to the last day of baseline ($t(9) = 6.63, p < 0.001$; $t(9) = 9.22, p < 0.01$). Throughout the stress period males ($14.14 \pm 1.18\%$) generally obtained significantly less pellets successfully when compared to females ($20.96 \pm 1.79\%$; $F(1,17) = 7.23, p < 0.05$). Female rats obtained more pellets successfully than males on day 6, 12 and 14 of stress (all t 's > 6.48 , all p 's < 0.05). On day 14 of stress, reaching success of females was twice as high as success of males.

During the post-stress period males had an overall significantly lower reaching success ($21.10 \pm 1.69\%$) than females ($36.98 \pm 2.47\%$; $F(1,17) = 11.78, p < 0.01$). In particular, males had lower success rates than females on most days of the recovery period (all t 's > -4.56 , all p 's < 0.05). Males reached an asymptote reaching success that was significantly lower than that of females. In addition, both males ($29.89 \pm 2.61\%$) and females ($45.50 \pm 6.56\%$) had significantly lower reaching success on the last day of post-stress when compared to the last day of baseline testing ($t(18) = 4.34, p < 0.0001$). Testing was terminated when no further improvements were recorded for 7 days on day 21 post-stress.

4.1.2. Number of attempts—There was no overall sex difference in the number of attempts per test session between male and female rats at baseline ($F(1,17) = 0.27, p = 0.61$). Overall, both males and females made more attempts at acute and chronic stress time points when compared to baseline ($t(9) = -4.98, p < 0.001$; $t(8) = -4.86, p < 0.001$; $t(9) = -6.26, p < 0.001$; $t(8) = -5.20, p < 0.001$; respectively; Fig. 2C). Compared to baseline, female animals also made significantly more attempts at the post-stress interval ($t(9) = -4.36, p < 0.01$). Furthermore, animals made less attempts during the post-stress interval when compared to the acute and chronic stress time points ($t(18) = 4.26, p < 0.001$; $t(1,18) = 4.11, p < 0.001$, respectively; Fig. 2C). However, overall animals still made significantly more attempts post-stress when compared to baseline ($t(18) = -2.59, p < 0.05$).

There was a significant sex difference during the restraint stress interval ($F(1,18) = 4.71, p < 0.05$). Specifically, males ($16.5 \pm 0.50\%$) made more attempts per day when compared to females ($14.1 \pm 0.93\%$) on day 6 and 12 of stress exposure ($t(18) = -2.34, p < 0.05$; $t(18) = 2.26, p < 0.05$, respectively; Fig. 2D). During the post-stress interval an overall significant sex difference was also present ($F(1,18) = 14.26, p < 0.01$). For instance, on the last day of post-stress testing males made significantly more attempts (13 ± 0.54) when compared to females (10.5 ± 1.22 ; $t(18) = 2.44, p < 0.05$; Fig. 2D).

4.1.3. Reaching total movement score—The qualitative analysis of reaching movements performed from video recordings taken at baseline and chronic stress time points revealed differences between sexes. Males had an overall significantly lower 35 point qualitative score when compared to females ($F(1,17) = 5.35, p < 0.05$; Fig. 3). There was no sex difference at specific time points. Animals had significantly lower movement scores at the chronic stress time point when compared to baseline ($t(19) = 6.46, p < 0.001$). Furthermore, all animals had significantly higher movement scores at the post-stress interval when compared to the chronic stress time point ($t(19) = -6.36, p < 0.001$).

4.1.4. Reaching subcomponent movement score—There was no overall sex difference in the reaching subcomponents at the baseline, chronic stress and post-stress time points. There were sex differences in individual movement components, however. After chronic stress, the grasp score of females was reduced when compared to males ($t(18) = -2.36, p < 0.05$). There also were a number of non-significant changes in various movement subcomponents in both sexes. The cumulative effect of these small changes might have affected reaching success. During the post-stress period, males had a significantly lower release score when compared to females ($t(18) = 4.28, p < 0.01$). These observations indicate that males required more extensive head and body movements to extract the pellet from the paw.

4.2. Skilled walking

4.2.1. Number of placement errors—There were non-significant trends of a time $\times$ sex interaction and overall sex difference (p 's > 0.05). In baseline, however, males (3.72 ± 1.08) made more than twice as many errors than females, although this difference was not significant (1.49 ± 0.71 ; $t(38) = 1.89, p = 0.07$; Fig. 4B). The trend for increased errors disappeared at both the chronic stress and post-stress time points.

4.2.2. Foot fault scoring—There were no differences in movement scores between males and females at baseline, chronic stress or post-stress test sessions ($F(1,16) = 2.69, p = 0.12$). There was, however, a significant difference in movement scores in animals tested 10 and 60 min after stress at the chronic stress time point ($F(1,35) = 6.66, p < 0.05$). Animals tested 10 min after restraint stress had significantly higher movement scores when compared to animals tested 60 min after stress ($t(38) = -2.15, p < 0.05$; Fig. 4C).

4.3. Open field activity

There was an overall difference in all aspects of open field behaviour between males and females during both baseline and chronic stress time points ($F(1,18) = 4.85, p < 0.05$; $F(1,18) = 7.13, p < 0.05$, respectively).

4.3.1. Vertical activity—At baseline and chronic stress time points, male rats showed less vertical activity than females ($t(18) = -2.83, p < 0.05$; $t(18) = -2.17, p < 0.05$, respectively; Fig. 5B).

4.3.2. Percent time in center—Females spent approximately 5% of the time in the center fields during baseline and chronic stress and 2% during the post-stress test sessions. Whereas males spent approximately 11% of the time in the center during baseline, their time measures were comparable to females at the other test sessions. There were no interactions or group differences, however.

4.3.3. Percent time in outside—Males spent approximately 88% and 97% of the time during baseline and chronic stress in the outside fields, respectively. Females spent approximately 94% of the time exploring outside fields during baseline and chronic stress and 98% during the post-stress test session. At the post-stress time point males spent the

same amount of time in the outside fields than females. There was no difference between the amount of time animals spent in the outside fields between and within groups.

4.3.4. Activity—Males entered approximately 62, 60 and 56 fields during baseline, chronic and post-stress time points, respectively. Females entered approximately 74, 87 and 79 fields. There were no differences between groups, however.

4.4. Corticosterone levels

There was an overall sex difference across the three time points ($F(1,16) = 5.09, p < 0.05$). Corticosterone levels were higher in males than in females at baseline ($t(18) = 2.21, p < 0.05$; Fig. 6A). At the chronic stress time point this difference had disappeared. At the same time, variance between subjects increased indicating greater inter-individual variety. Females had significantly higher corticosterone levels than males at the post-stress time point ($t(16) = -3.46, p < 0.01$).

4.5. Glucose measurements

There was no sex difference in glucose measurements taken at baseline and chronic stress time points ($F(1,18) = 0.94, p = 0.35$). The post-stress measurement revealed that males had higher glucose concentrations than females ($t(17) = -2.48, p < 0.05$; Fig. 6B).

4.6. Body weight

Percent baseline body weight analysis revealed that males and females did not show significant differences at the chronic stress time point ($F(1,18) = 3.64, p < 0.07$). There were also no significant within-animal differences. During the post-stress period, however, males and females were significantly different ($F(1,17) = 5.96, p < 0.05$). Males had higher weight gain than females during the first 2 weeks of post-stress testing.

5. Discussion

The objective of the present study was to compare stress-induced changes in motor function in male and female rats. The experiment used a standard stress model and a high-resolution behavioural test battery to obtain quantitative and qualitative measures of fine motor performance. The behavioural tests revealed that acute and chronic restraint stress alters both skilled and non-skilled motor performance in males and females. Sex differences in stress sensitivity were observed in skilled reaching and vertical open field exploration, but not in skilled walking. Interestingly, sex differences were also found in the recovery from stress-induced motor disturbances. While males showed generally reduced reaching accuracy and needed more attempts to grasp a pellet, they also recovered more slowly from stress-induced impairments.

The present stress-induced alterations in skilled limb use resemble those described earlier [16,17,69]. Acute and chronic restraint stress led to reduction in reaching success along with an increase in number of attempts needed to grasp a pellet. These findings indicate reduced accuracy of skilled movements in animals exposed to stress. The behavioural impairments associated with stress, however, were not necessarily accompanied by corticosterone

elevation. The presence of movement impairments in the absence of elevated corticosterone levels is in line with previous work [16] suggesting that stress-induced motor impairments might be independent of corticosterone. This is further supported by absent effects of the interval between stress and behavioural test session (i.e., 10 min versus 60 min) and by the finding of faster recovery of post-stress reaching success in females despite higher corticosterone levels. Furthermore, the observation that higher corticosterone measurements were not always linked to higher glucose levels and the lack of stress effects on body weight might indicate that the stress regimen represented a rather mild stressor.

When considering the present behavioural data in the light of physiological variables, it is important to note that exercise-related factors might have influenced circulating corticosterone levels in males at baseline. For instance, skilled reach training may have altered corticosterone secretion, thus disconnecting behavioural and physiological variables. Performing a demanding task such as skilled reaching might represent an exercise regimen that by itself elevates glucocorticoid levels, as shown for other kinds of physical activity [30,72]. Furthermore, it is also important to note that handling, exposure to an unfamiliar environment and anesthesia associated with the blood sampling procedure might have modified HPA axis activity. A previous study indicated that anesthesia in particular might affect plasma corticosterone levels [31]. Although Vahl et al. [31] referred to pentobarbital injection, the inhalational anesthesia used in the present study might still present a significant influence on corticosterone levels.

Although there was a trend for female rats to acquire the reaching task faster than males, there was no sex difference in overall reaching success and the number of attempts made at baseline. Since Field and Whishaw [3] reported that males and females make different postural adjustments prior to reach movement initiation, it is possible that differential pre-reach adjustments influenced reaching movement performance during stress. In particular, one would expect that especially postural changes accompanying a reach determine the ability to compensate for distal impairments. Because compensatory adjustments frequently involve greater body displacement during reaching, the greater displacement shown by Field and Whishaw [3] in females might present an advantage in promoting reaching success when motor function is compromised. Although the presently used movement score included measures of posture during reaching, the sampled components might not have been sensitive enough to capture discrete postural differences that might have affected reaching success of males and females.

In contrast to baseline, exposure to chronic restraint stress did uncover sex differences in these parameters. Although both sexes were equally impaired on the first day of stress, as exposure to stress continued, females began to improve more steadily than males. Consequently, success rates and number of attempts began to diverge. These data are in contrast to reports of female Wistar rats showing greater locomotor effects and physiological susceptibility to chronic mild stress, such as restraint [32,33]. This discrepancy might derive from strain- and colony-dependent behavioural and physiological differences. In general, locomotor activity in Long-Evans females was described as being more stable than male activity [13]. Nevertheless, the present findings of impaired reaching success in both genders emphasize the sensitivity of the skilled reaching task to detect stress-induced motor

disturbance. Furthermore, changes in stress-exposed rats might be task-specific. The narrow opening of the skilled pellet reaching apparatus restricts the degree of freedom in movement and so the use of compensatory adjustments becomes limited [34]. Thus, decreased success rates and increased reaching attempts might directly reflect deviations in limb trajectories or body position in front of the opening [34].

The stress regimen used in the present study also had a pronounced effect on motor activity. Open field exploratory activity is thought to serve as indicator of stress-associated emotional reactivity [35,36]. There is a large variety in the effects of stress on locomotor activity described in the literature. While an acute stressor might produce hyperactivity, as reflected by elevated horizontal and vertical activity [37], it can at the same time also reduce exploration in male rats [13]. The present findings show enhanced exploratory activity in female rats during baseline and after chronic stress. Recovery from stress, in turn, led to decreased activity levels in females, although a trend for sex difference still existed.

A particularly interesting finding of the present study is the observation of sex differences in the recovery from stress. The present data show that sex did not affect recovery from acute stress, however, an interesting finding is the observation that restraint stress exerts its effects slowly i.e., 60 min after the stress session, at a time when the peak of corticosterone elevation has passed [16,38]. Again, this observation supports the notion that stress might affect motor system function independently of elevated corticosterone levels. However, it is important to acknowledge that elevated corticosterone levels are part of a complex physiological cascade [39] so that the present findings might be related to a component other than this particular hormone.

The largest difference between males and females in recovery was seen in post-stress recovery. Male rats might have experienced greater difficulty habituating to the disturbing effects of chronic stress and so lacked successful compensatory movement strategies. This is suggested by their rather preserved qualitative movement patterns compared to females. Males showed a lower reaching movement score than females, which might reflect deviations in the original movement pattern in order to develop adaptive compensatory strategies. This relationship might explain the observation that higher success rates in females were accompanied by lower movement scores.

Although at asymptotic levels, neither males nor females reached their former baseline success rates in post-stress recovery. Males, however, reached at overall lower success rates than females. These findings are in line with observations that effects of exposure to chronic restraint stress can be long lasting. For example, animals exposed to chronic restraint for 3 weeks still showed enhanced anxiety 3 weeks after cessation of stress [39]. Although many stress-induced morphological and neurochemical changes may persist (e.g. [40]), others such as apical dendritic retraction may be reversed within 3 weeks after stress [41]. Administration of anxiolytic or antidepressant drugs, however, might be able to reverse both anatomical and behavioural changes caused by chronic stress [42,43]. For example, treatment with alcohol or diazepam can significantly improve skilled reaching performance and alleviate motor impairments associated with stress [16,44]. Similar findings were made applying antidepressant drugs to alleviate stress-induced spatial memory impairments [42].

The present data demonstrate that chronic restraint stress is associated with characteristic motor impairments depending on gender. Differences in motor performance might be related to different levels of anxiety [16,32]. Since chronic restraint stress modulates anxiety [45], motor performance may become altered by activation of the arousal response [46], which involves the amygdala and its connections to the prefrontal–striatal system and nucleus accumbens [47]. Moreover, it is also possible that stress-induced alterations in catecholaminergic and serotonergic activity affect fine motor control and contribute to decreased reaching success [17,48,49]. Because these neurotransmitters function as modulators of motor neuron excitability, elevated neuroendocrine activity during heightened arousal has been implicated as a factor that can alter motor output [50]. Interestingly, the dopaminergic system in males seems particularly vulnerable to disturbing events such as lesions or aging [51,52]. It was hypothesized that locomotor activity in male and female rats is differentially modulated by D1 and D2 dopamine receptors [53]. These data support the present findings and suggest that the male motor system, at least in part, exhibits a greater response to stress possibly due to higher sensitivity of the dopaminergic system.

It is generally believed that females react more robustly to stress, both behaviourally and physiologically [13,33,54]. One reason for this sex difference is that HPA axis activity occurs as a function of ovarian cycle stage. For example, females have a stronger reaction to a stressor during the proestrus stage of the estrous cycle [7,12,55]. Estrous data collection was not incorporated into the present study, however, many studies have concluded that estrogen is a key factor for sex differences in response to stress [7,12,56]. For example, estradiol [57] or estrogen [56] treatment enhances corticosterone release in response to stress. While a previous study reported motor function to be more susceptible to arousal in women than in men [46], the physiological stress response usually seems to be greater in males [58,59].

Sex differences like the ones described here have also been found in a number of other behavioural parameters. The literature suggests major sex differences in stress effects on cognition and emotion [7,60,61]. For example, acute stress has been shown to impair spatial memory in males but not females [7]. In fact, acute stress might even facilitate memory processes regardless of their stage in estrous cycle [7]. Interestingly, Paré et al. [60] proposed that females are more susceptible to chronic stress than males. Furthermore, under certain circumstances, males and females might even respond in opposite directions to similar experiences. In response to an acute stressful experience, male rats acquire an associative learning task faster [62,63], whereas female rats exposed to the same stimulus are impaired and show fewer conditioned responses [64–66].

6. Conclusion

The present study is the first to explore stress-induced sex differences in motor system function. The findings show that acute and chronic stress disturbs movement performance in both male and female rats, although different aspects of movement are affected. The present data identify discrete movement alterations characteristic for either male or female animals. Movement patterns and recovery from stress suggest that males and females might be equally susceptible to stress, however, they use different strategies to overcome stress-

induced motor disturbance. While male rats prefer to use original movement patterns, females tend to modify these patterns in order to increase reaching success. The latter strategy proves more effective in the recovery from stress because females show a faster rate of motor improvement. These findings provide insight into possible influences of stress on functional recovery from brain damage in males and females.

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References

1. Field, EF., Pellis, SM. Sex difference in the organization of behaviour patterns: endpoint measures do not tell the whole story. In: Ellis, L., Ebertz, L., editors. *Males, females, and behaviour*. London: Praeger; 1998.
2. Field EF, Metz GA, Pellis SM, Whishaw IQ. Sexually dimorphic postural adjustments during vertical behaviour are altered in a unilateral 6-OHDA rat model of Parkinson's disease. *Behav Brain Res*. 2006; 174:39–48. [PubMed: 16930735]
3. Field EF, Whishaw I. Sexually dimorphic postural adjustments are used in a skilled reaching task in the rats. *Behav Brain Res*. 2005; 163:237–45. [PubMed: 16023226]
4. Field EF, Whishaw IQ, Pellis SM. Sex differences in catalepsy: evidence for hormone-dependent postural mechanisms in haloperidol-treated rats. *Behav Brain Res*. 2000; 109:207–12. [PubMed: 10762690]
5. Bowman RE, Zrull MC, Luine VN. Chronic restraint stress enhances radial arm maze performance in female rats. *Brain Res*. 2001; 904:279–89. [PubMed: 11406126]
6. Bowman RE, Ferguson D, Luine VN. Effects of chronic restraint stress and estradiol on open field activity, spatial memory, and monoaminergic neurotransmitters in ovariectomized rats. *Neuroscience*. 2002; 113:401–10. [PubMed: 12127097]
7. Conrad C, Jackson J, Wiczorek L, Baran S, Harman J, Wright R, et al. Acute stress impairs spatial memory in male but not female rats: influence of estrous cycle. *Pharmacol Biochem Behav*. 2004; 78:569–79. [PubMed: 15251266]
8. Kitraki E, Kremmyda O, Youlatos D, Alexis MN, Kittas C. Gender-dependent alterations in corticosteroid receptor status and spatial performance following 21 days of restraint stress. *Neuroscience*. 2004; 125:47–55. [PubMed: 15051144]
9. Frankenhaeuser M, Dunne E, Lundberg U. Sex differences in sympathetic adrenal medullary reactants induced by different stressors. *Psychopharmacology*. 1976; 47:1. [PubMed: 959463]
10. Haleem D, Kennett G, Curzon G. Adaptation of female rats to stress: shift to male pattern by inhibition of corticosterone synthesis. *Brain Res*. 1988; 458:339–47. [PubMed: 2463050]
11. Campbell T, Lin S, DeVires C, Lambert K. Coping strategies in male and female rats exposed to multiple stressors. *Physiol Behav*. 2003; 78:495–504. [PubMed: 12676287]
12. Carey M, Deterd C, Koning JD, Helmerhorst F, de Kloet ER. The influence of ovarian steroids on the hypothalamic–pituitary–adrenal regulation in the female rat. *J Endocrin*. 1995; 144:311–21.
13. Faraday M. Rat sex and brain differences in responses to stress. *Physiol Behav*. 2002; 75(4):507–22. [PubMed: 12062315]
14. Luine V. Sex differences in chronic stress effects on memory in rats. *Stress*. 2002; 5:205–16. [PubMed: 12186683]
15. Metz G, Schwab M, 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]

16. Metz G, Jadavji N, Smith L. 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]
17. Metz G. Stress as a modulator of motor system function and pathology. *Rev Neurosci.* 2007; 18:1–14. [PubMed: 17405448]
18. Garcia A, Marti O, Valles A, Dal-Zotto S, Armario A. Recovery of the hypothalamic–pituitary–adrenal response to stress. Effect of stress intensity, stress duration and previous stress exposure. *Neuroendocrin.* 2000; 72:114–25.
19. Mercier S, Frederic, Canini, Buguet A, Cespuglio R, Martin S, et al. Behavioural changes after an acute stress: stressor and test types influences. *Behav Brain Res.* 2003; 139:167–75. [PubMed: 12642187]
20. 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]
21. Metz GA, Whishaw IQ. Drug-induced rotation intensity in unilateral dopamine-depleted rats is not correlated with end point or qualitative measures of forelimb or hindlimb motor performance. *Neuroscience.* 2002; 111:325–36. [PubMed: 11983318]
22. Eshkol, N., Wachmann, A. Movement notation. London, UK: Weidenfeld and Nicolson; 1958.
23. Whishaw IQ, Pellis SM. The structure of skilled forelimb reaching in the rat: a proximally driven movement with a single distal rotatory component. *Behav Brain Res.* 1990; 41:49–59. [PubMed: 2073355]
24. 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]
25. Metz GA, Whishaw IQ. Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. *J Neurosci Methods.* 2002; 115:169–79. [PubMed: 11992668]
26. Suchecki D, Duarte Palma B, Tufik S. Pituitary-adrenal axis and behavioural responses of maternally deprived juvenile rats to the open field. *Behav Brain Res.* 2000; 111:99–106. [PubMed: 10840136]
27. Ishida H, Mitsui K, Nukaya H, Matsumoto K, Tsuji K. Study of active substances involved in skin dysfunction induced by crowding stress. Effect of crowding and isolation on some physiological variables, skin function and skin blood perfusion in hairless mice. *Biol Pharma Bull.* 2003; 26:170–81.
28. Kavushansky A, Richter-Levin G. Effects of stress and corticosterone on activity and plasticity in the amygdala. *J Neurosci Res.* 2006; 84:1580–7. [PubMed: 16998919]
29. Colazo MG, Ambrose DJ, Kastelic JP, Small JA. Comparison of 2 enzyme immunoassays and a radioimmunoassay for measurement of progesterone concentrations in bovine plasma skim milk, and whole milk. *Can J Vet Res.* 2008; 72:32–6. [PubMed: 18214159]
30. Adlard PA, Cotman CW. Voluntary exercise protects against stress-induced decreases in brain-derived neurotrophic factor protein expression. *Neuroscience.* 2004; 124:985–92. [PubMed: 15026138]
31. Vahl TP, Ulrich-Lai YM, Ostrander MM, Dolgas CM, Elfers EE, Seeley RJ, et al. Comparative analysis of ACTH and corticosterone sampling methods in rats. *Am J Physiol Endocrinol Metab.* 2005; 289:E823–828. [PubMed: 15956051]
32. Dalla C, Antoniou K, Drossopolou G, Xagoratis M, Kokras N, Sfikakis A, et al. Chronic mild stress impact: are females more vulnerable? *Neuroscience.* 2005; 135(3):703–14. [PubMed: 16125862]
33. Mashoodh R, Wright LD, Hebert K, Perrot-Sinal TS. Investigation of sex differences in behavioural, endocrine, and neural measures following repeated psychological stressor exposure. *Behav Brain Res.* 2008; 188:368–79. [PubMed: 18179829]
34. Kirkland SW, Smith LK, Colwell KL, Metz GA. Chronic stress influences motor recovery and compensation following focal ischemic stroke in a rat model. *Brain Res.* submitted for publication.

35. Levine S, Glick D, Nakane PK. Adrenal and plasma corticosterone and vitamin A in rat adrenal glands during postnatal development. *Endocrinology*. 1967; 80:910–4. [PubMed: 4290303]
36. Crawley JN. Exploratory behavior models of anxiety in mice. *Neurosci Biobehav Rev*. 1985; 9:37–44. [PubMed: 2858080]
37. Windle RJ, Shanks N, Lightman SL, Ingram CD. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. *Endocrin*. 1997; 138:2829–34.
38. Sakakura M, Saito Y, Takebe K, Yamashita I, Ishii K. Time course of hypothalamic CRF activity after the administration of two different stresses. *Endocrinol Jpn*. 1976; 23:413–6. [PubMed: 1087604]
39. Vyas A, Chattarji S. Modulation of different states of anxiety-like behavior by chronic stress. *Behav Neurosci*. 2004; 118:1450–4. [PubMed: 15598155]
40. Pham K, Nacher J, Hof PR, McEwen BS. Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult rat dentate gyrus. *Eur J Neurosci*. 2003; 17:879–86. [PubMed: 12603278]
41. Radley JJ, Morrison JH. Repeated stress and structural plasticity in the brain. *Ageing Res Rev*. 2005; 4:271–87. [PubMed: 15993654]
42. Luine V, Villegas M, Martinez C, McEwen BS. Repeated stress causes reversible impairments of spatial memory performance. *Brain Res*. 1994; 639:167–70. [PubMed: 8180832]
43. Wood GE, Young LT, Reagan LP, Chen B, McEwen BS. Stress-induced structural remodeling in hippocampus: prevention by lithium treatment. *Proc Natl Acad Sci USA*. 2004; 101:3973–8. [PubMed: 15001711]
44. Metz GA, Gonzalez CL, Piecharka DM, Whishaw IQ. Acute alcohol administration improves skilled reaching success in intact but not 6-OHDA dopamine depleted rats: a subsystems analysis of the motoric and anxiolytic effects of alcohol. *Behav Brain Res*. 2003; 142:167–714. [PubMed: 12798278]
45. Nosek K, Dennis K, Andrus BM, Ahmadiyeh N, Baum AE, Solberg Woods LC, et al. Context and strain-dependent behavioral response to stress. *Behav Brain Funct*. 2008; 4:23. Epub ahead of print. [PubMed: 18518967]
46. Noteboom JT, Fleshner M, Enoka RM. Activation of the arousal response can impair performance on a simple motor task. *J Appl Physiol*. 2001; 91:821–31. [PubMed: 11457799]
47. Groenewegen HJ, Wright CI, Beijer AV. The nucleus accumbens: gateway for limbic structures to reach the motor system? *Prog Brain Res*. 1996; 107:485–511. [PubMed: 8782538]
48. Kennett G, Chaouloff F, Marcou M, Curzon G. Female rats are more vulnerable than males in an animal model of depression: the possible role of serotonin. *Brain Res*. 1986; 382:416–21. [PubMed: 3756525]
49. Rivier C. Gender, sex steroids, corticotropin-releasing factor, nitric oxide, and the HPA response to stress. *Pharmacol Biochem Behav*. 1999; 64:739–51. [PubMed: 10593197]
50. Katz PS, Frost WN. Intrinsic neuromodulation: altering neuronal circuits from within. *Trends Neurosci*. 1996; 19:54–61. [PubMed: 8820868]
51. Cass WA, Peters LE, Smith MP. Reductions in spontaneous locomotor activity in aged male, but not female, rats in a model of early Parkinson's disease. *Brain Res*. 2005; 1034:153–61. [PubMed: 15713267]
52. Tamás A, Lubics A, Lengvári I, Reglodi D. Effects of age, gender, and gonadectomy on neurochemistry and behavior in animal models of Parkinson's disease. *Endocrine*. 2006; 29:275–87. [PubMed: 16785603]
53. Schindler CW, Carmona GN. Effects of dopamine agonists and antagonists on locomotor activity in male and female rats. *Pharmacol Biochem Behav*. 2002; 72:857–63. [PubMed: 12062575]
54. Kandandrea D, Kittas C, Kitraki E. Forced swimming differentially affects male and female brain corticosteroid receptors. *Neuroendocrin*. 2002; 75:217–26.
55. Phillips JG, Poolsanguan W. A method to study temporal changes in adrenal activity in relation to sexual status in the female laboratory rat. *J Endocrinol*. 1978; 77:283–91. [PubMed: 207813]
56. Handa R, Nunley K, Lorena S, Louie J, McGivern R, Bollnow M. Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors. *Physiol Behav*. 1994; 55:117–24. [PubMed: 8140154]

57. Figueiredo HF, Ulrich-Lai YM, Choi DC, Herman JP. Estrogen potentiates adrenocortical responses to stress in female rats. *Am J Physiol Endocrinol Met.* 2007; 292:E1173–1182.
58. Collins A, Frankenhaeuser M. Stress responses in male and female engineering students. *J Hum Stress.* 1978; 4:43–8.
59. Kirschbaum C, Wüst S, Hellhammer D. Consistent sex differences in cortisol responses to psychological stress. *Psychosom Med.* 1992; 54:648–57. [PubMed: 1454958]
60. Paré W, Blair G, Kluczynski J, Tejani-Butt S. Gender differences in acute and chronic stress ins Wistar Kyoto rats. *Int Physiol Behav Sci.* 1999; 34:227–41.
61. Renard GM, Suarez MM, Levin GM, Rivarola MA. Sex differences in rats: effects of chronic stress on sympathetic system and anxiety. *Physiol Behav.* 2005; 85:363–9. [PubMed: 15939444]
62. Shors TJ, Weiss C, Thompson RF. Stress-induced facilitation of classical conditioning. *Science.* 1992; 257:537–9. [PubMed: 1636089]
63. Shors T, Chua C, Falduto J. Sex differences and opposite effects on stress on dendritic spine density in the male versus female hippocampus. *J Neurosci.* 2001; 21:6292–7. [PubMed: 11487652]
64. Wood GE, Shors TJ. Stress facilitates classical conditioning in males, but impairs classical conditioning in females through activational effects of ovarian hormones. *Proc Natl Acad Sci USA.* 1998; 95:4066–71. [PubMed: 9520494]
65. Shors TJ, Lewczyk C, Pacynski M, Mathew PR, Pickett J. Stages of estrous mediate the stress-induced impairment of associative learning in the female rat. *Neuroreport.* 1998; 9:419–23. [PubMed: 9512383]
66. Wood GE, Beylin AV, Shors TJ. The contribution of adrenal and reproductive hormones to the opposing effects of stress on trace conditioning in males versus females. *Behav Neurosci.* 2001; 115:175–87. [PubMed: 11256441]
69. Kirkland SW, Coma A, 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]
72. Rojas Vega S, Strüder HK, Vera Wahrmann B, Schmidt A, Bloch W, Hollmann W. Acute BDNF and cortisol response to low intensity exercise and following ramp incremental exercise to exhaustion in humans. *Brain Res.* 2007; 1121:59–65.

Fig. 1.

Time chart illustrating the order of manipulations and behavioural tests. Daily training and testing included the skilled reaching task. Skilled reaching, skilled walking and open field activity performance were assessed from video recordings collected before the stress treatment (baseline), at chronic stress and chronic post-stress time points. Blood samples for corticosterone and glucose analyses were collected at baseline, chronic stress and post-stress time points as indicated.

Fig. 2.

Quantitative skilled reaching performance before, during and after restraint stress. (A) Photograph illustrating the skilled reaching task in which rats were required to grasp and retrieve individual food pellets. (B) Time course of reaching success. There was a significant reduction in percent success from the last day of baseline testing to the first day of stress. Also note significant sex differences during stress and during the post-stress testing periods. (C) Overall number of attempts to grasp a single food pellet during stress in males and females. Note that acute (day 1) and chronic stress (day 14) caused a significant increase in the number of attempts when compared to baseline for both sexes. (D) Time course of number of attempts in male versus female rats. Note significant sex differences throughout the stress and post-stress time points. Asterisks indicate significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, unpaired t -test comparison between males and females (B and D) and paired t -test comparison between animals at baseline and stress time points (C). \$\$\$ $p < 0.001$ comparison to baseline testing (B).

Fig. 3.

Qualitative skilled reaching performance. Reaching movement score was assessed by a detailed 35-point scale before (baseline) and after 14 days of restraint stress (chronic stress), and at the end of the stress recovery period (post-stress). Note the overall higher movement scores in male rats. Asterisk indicates significance: $*p < 0.05$, ANOVA overall difference between males and females.

Fig. 4.

Skilled walking task. (A) Photograph illustrating a rat crossing the ladder rung walking apparatus. (B) Number of errors at baseline. There was a trend of higher error numbers in male rats as compared to females. (C) Movement scores at chronic stress time point. Note animals tested 10 min after stress had a significantly higher movement score than animals tested 60 min after stress. Asterisks indicate significance levels: $*p < 0.05$, unpaired t -test comparison between 10- and 60-min groups.

Fig. 5. Open field activity. (A) Photograph illustrating the open field task. (B) Number of rears in an open field. Note the significantly increased number of rears at baseline and after 14 days of restraint stress. Asterisks indicate significance: * $p < 0.05$, unpaired t -test comparison between males and females.

Fig. 6. Plasma corticosterone (A) and glucose (B) concentrations prior to stress (baseline), after 15 days of daily exposure to restraint stress and after 21 days of post-stress testing. Note that males had significantly higher corticosterone levels during baseline, however, female corticosterone levels were higher in females at post-stress testing. Furthermore, males had significantly higher plasma glucose levels when compared to females. Asterisks indicate significances: * $p < 0.05$; ** $p < 0.01$, unpaired t -test comparison between males and females.