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Effects of Training on Stress-Related Behavior of the Common Marmoset (Callithrix jacchus) in Relation to Coping With Routine Husbandry Procedures

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Using positive reinforcement, J. McKinley trained 12 common marmosets (Callithrix jacchus) to provide urine samples on request. The study then exposed the marmosets to mildly stressful, routine husbandry procedures (i.e., capture and weighing). The nonhuman animals spent less time inactive poststressor as opposed to prestressor. L. Bassett collected matched behavioral data from 12 nontrained marmosets who were less accustomed to human interaction. These animals spent significantly more time self-scratching and locomoting as well as less time inactive, poststressor. Collapsed data from the 2 populations showed increased scent marking, poststressor. These results suggest that locomotion, self-scratching, and scent marking are useful, noninvasive behavioral measures of stress and, thus, reduced welfare in the common marmoset. Overall, nontrained animals showed more self-scratching than did their trained counterparts. It was not possible to collect urine from nontrained marmosets. In response to the stressor, however, trained animals showed no significant change in excreted urinary cortisol. These results suggest that training marmosets may allow them to cope better with routine laboratory procedures.

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The common marmoset (*Callithrix jacchus*) is used extensively in behavioral (Williams, 1987) and biomedical (Hearn, Abbott, Chalmers, Hodges, & Lunn, 1978) research. Despite this, there has been a paucity of studies attempting to identify behaviors associated with increased or decreased welfare, resulting from the captive environment, husbandry procedures, or experimental manipulations. Johnson et al. (1996) found an increase in plasma cortisol in this species, in response to isolation, to be associated with increased movement, which was interpreted as an indicator of behavioral arousal. In contrast, increases in plasma cortisol because of housing in an unstable peer group were associated with increases in aggressive and submissive behaviors related to agonistic encounters.

Orally administered anxiety-reducing benzodiazepine drugs have been shown to reduce the frequency of self-scratching in the common marmoset (Cilia & Piper, 1997), suggesting that this behavior may be associated with stress. In the same study, the anxiolytic drugs resulted in decreases in scent marking and aggressive behavior. Increases in allogrooming also were seen following drug administration, indicating that muscle relaxation was not responsible for the decreases in the other behaviors seen. Anxiolytic drugs did not, however, affect rates of locomotion. This suggests, in contrast to the study by Johnson et al. (1996), that locomotory behavior is unrelated to anxiety.

Increased activity in the hypothalamic-pituitary-adrenal (HPA) axis in response to physical or psychological challenge results in elevated circulatory glucocorticoids such as cortisol. Various species of primates show increases in plasma cortisol in response to stressors such as restraint (Reinhardt, Liss, & Stevens, 1995), exposure to high intensity noise (Hanson, Larson, & Snowdon, 1976), and maternal separation (Hennessy, 1997). Studies also have assayed urine for cortisol concentrations (Crockett, 1998). Use of urine or saliva as opposed to blood has the advantage in that it may be collected noninvasively. Blood collection procedures involving capture; restraint; and, possibly, anesthesia may, themselves, result in elevated cortisol levels, affecting experimental results. Weid’s black, tufted-ear marmosets (*Callithrix kuhli*), trained to give urine samples on request, show significantly elevated levels of urinary cortisol following a stressor such as isolation in a small cage for 11 hr (Smith & French, 1997).

This study aimed to validate the use of both behavior and urinary cortisol as reliable and sensitive measures of stress and, therefore, welfare in the common marmoset. Both behavioral and physiological measures may be useful as welfare indicators (Duncan & Fraser, 1997; Mason & Mendl, 1993). A demonstrable positive or negative correlation between urinary cortisol concentration and frequency of a particular behavior following a stressor will increase the validity of the use of changes in frequency of the behavior as an effective indirect welfare indicator (Mason & Mendl, 1993). Prior experience of positive handling affects responses to stressors in many species of animal, and taming may reduce the physiological activity of the HPA axis (Grandin, 1997). Therefore, this study
also sought to assess the effects of training in relation to welfare and coping with routine laboratory procedures.

METHOD

Study Animals

The study animals were 24 common marmosets—12 males and 12 females—with a mean age of 1,089 days (± SE 135.67) as of January 2, 2001. Animals in the training group (n = 12 animals) had a mean age of 1,188 days (± SE 232.37 days) and those in the nontraining group (n = 12 animals), a mean age of 989 days (± SE 145.55 days). The ages of animals in the two groups were not significantly different from each other, \( t(12) = 0.72; p = .13 \).

The marmosets were housed in male–female pairs at the Medical Research Council (MRC) Human Reproductive Sciences Unit, Edinburgh, Scotland. See McKinley, Buchanan-Smith, Bassett, and Morris (2003/this issue) for details of housing. Animals in upper and lower tier cages were balanced between conditions. All trained marmosets were housed in cages within the same colony room, and nontrained animals in an adjacent room. None of the females in the study were past the first trimester of pregnancy, as detected by transabdominal uterine palpations, which were performed regularly. This generally is considered a reliable method for detecting pregnancy in this species (Hearn et al., 1978) and was important as cortisol levels may be affected by pregnancy (Bazer, 1998). Over a period of approximately 6 weeks, McKinley (McKinley et al., 2003/this issue) trained animals in the training group to provide urine samples for analysis. Animals in the nontraining group were not trained and were not exposed to any additional positive human interaction.

Experimental Procedure

On the day of the stressor, each of the animals was chased into the nestbox, which then was closed and removed from the cage. The nestbox was taken into a separate room in which the marmosets were removed one at a time and transferred by gloved hand to a small cage to be weighed. They then were returned to the nestbox, which was replaced in the homecage and opened to allow the animals to re-enter the homecage at will. The whole procedure took between 4 min and 4 min, 30 sec for trained animals (mean time 4 min, 9 sec; ± SE 4.73 sec) and 3 min, 45 sec and 4 min, 30 sec (mean time 4 min, 14 sec; ± SE 7.24 sec) for nontrained animals. The amount of time spent away from the homecage was not significantly different for animals in training and nontraining groups, \( t(10) = \)
The stressors were administered on March 7, 2001 and March 14, 2001 (both Wednesdays) between 0930h and 1030h. Removal from the homecage for weighing is a standard laboratory procedure and is carried out several times a year.

Cortisol Enzyme Immunoassay

T. E. Smith (then of Queen’s University, Belfast, Ireland) measured cortisol concentrations in all urine samples. The enzyme immunoassay was validated immunologically as described by Reimers, Salerno, and Lamb (1996). Serial dilutions of four urine pools gave parallel displacement curves with a standard solution. This confirmed that the cortisol in the urine samples was identical immunologically with standard cortisol preparations (from Sigma Chemical Company). Recovery of known amounts of cortisol standard (n = 5 stds: 500, 250, 125, 62.5, 31.25 pg/50ul) from high and low concentrations of a urine pool had a mean of 80.83 ± SE 1.9 (n = 3 repeats for high pool and 3 repeats for low pool). Intra-assay coefficients of variation for high and low concentration pools were 4.68% and 1.91%, respectively (n = 11). Inter-assay coefficients of variation for high and low concentration pools were 9.30% and 14.89%, respectively (n = 11). Sensitivity was 1.95 pg/50ul, equivalent to 39 pg/1ml. To correct for urine dilution, creatinine concentrations were quantified for each sample (Tietz, 1976) and cortisol expressed as µg cortisol/mg Cr/ml.

Behavioral Data Collection and Statistical Analysis

Urine was collected immediately after the behavioral data were recorded. Scan sampling was used with an interval of 15 sec between scans; data collection sessions lasted for 5 min. Data were collected on a palm top computer using The Observer 3.0 software (Noldus, 1993). The recorded behaviors were mutually exclusive and included “Inactive,” “Locomote,” “Self-Scratch,” “Scent Mark,” “Vocalize,” and “Forage” (see Table 1).

An “other” category also was used and included behaviors infrequently seen, such as “allogrooming” and “inactive, inalert” behavior. One set of prestressor data was recorded for each monkey (trained and nontrained) at each of three time periods, 1200h, 1400h, and 1600h. Matching data were collected for both groups following administration of the stressor. No significant main effects were found for vocalizing or foraging; therefore, these data are not discussed further. Data were found to be normally distributed throughout; hence, parametric tests were used. A two-factor, within-subjects analysis of variance (ANOVA) was used to test for effect of stress condition (pre- and poststressor) and time of day (1200h, 1400h, and 1600h) on uri-
nary cortisol concentrations. A two-factor, repeated-measures ANOVA was carried out to determine whether behavior changed over time following the stressor (on the day that the stressor was administered). The factors analyzed were stress and time period as well as the interaction between the two. Separate analyses were carried out initially for trained and nontrained animals.

After this, a three-factor mixed ANOVA was carried out using behavioral data from both groups of animals. This was to see if there was an effect of training on behavior and to increase the sample size effectively by combining both sets of data. The variables analyzed were stress, time period, and training.

Significance was set at $\alpha < 0.05$ throughout the analyses. Where significant main effects were found using repeated-measures ANOVAs, where appropriate, post-hoc pairwise $t$ tests with the Bonferroni correction were used. These were intended to pinpoint in which differences lay, while controlling against Type II errors. For behavioral data, to ensure statistical independence, a single mean was calculated from both animals in a pair. Each pair, therefore, was effectively treated as one individual in the analysis. Data used consisted of mean sample points per session; 20 sample points were obtained per pair per 5 min session.

RESULTS

Behavioral Analyses

For trained animals, there was significantly less Inactive after the stressor compared with before it (see Table 2 and Figure 1). There also was an effect of time period on Inactive behavior, with animals being more Inactive before the stressor than after it. For nontrained animals, there was no significant effect of stress or time period on Inactive behavior.
of observation on Locomote (see Table 2). However, no significant differences between the individual observation times were found. There were no significant interactions between the variables of stressor and time for any of the behaviors (see Table 2).

For nontrained animals, there was significantly less Inactive behavior after the stressor compared with before it (see Figure 1). There was significantly more Self-Scratch behavior after the stressor compared with before it (see Figure 1). There was no effect of time of observation on behavior (see Table 3). The only behaviors that showed a significant interaction between time and stressor were Inactive and Self-Scratch (see Table 3; see Figures 2 and 3). Levels of Inactivity remained relatively stable over time for the prestressor condition; after the stressor, they were reduced dramatically at 1200h. At 1400h, poststressor levels had risen slightly. They

### Table 2

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Stressor</th>
<th>Time</th>
<th>Stressor × Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F(1, 5)</td>
<td>p</td>
<td>F(2, 10) p</td>
</tr>
<tr>
<td>Inactive</td>
<td>36.14</td>
<td>&lt; .01</td>
<td>3.05 .09</td>
</tr>
<tr>
<td>Locomote</td>
<td>4.00</td>
<td>.10</td>
<td>4.37 &lt; .05</td>
</tr>
<tr>
<td>Self-Scratch</td>
<td>0.63</td>
<td>.47</td>
<td>2.25 .16</td>
</tr>
<tr>
<td>Scent Mark</td>
<td>4.22</td>
<td>.10</td>
<td>1.34 .31</td>
</tr>
</tbody>
</table>

![Figure 1](image)

**FIGURE 1** Mean sample points spent performing each behavior pre- and poststressor, for trained and nontrained animals (collapsed across 1200h, 1400h, and 1600h; bars represent standard errors).
### Table 3
Results of Within-Subjects Analysis of Variances of Effects of Stressor, Time of Observation and the Interaction Between the Two Variables on All Behaviors for Nontrained Animals

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Stressor $F(1, 5)$</th>
<th>Stressor $p$</th>
<th>Time $F(2, 10)$</th>
<th>Time $p$</th>
<th>Stressor × Time $F(2, 10)$</th>
<th>Stressor × Time $p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive</td>
<td>33.06</td>
<td>&lt; .01</td>
<td>3.16</td>
<td>.09</td>
<td>4.72</td>
<td>&lt; .05</td>
</tr>
<tr>
<td>Locomote</td>
<td>3.57</td>
<td>.12</td>
<td>0.62</td>
<td>.56</td>
<td>0.67</td>
<td>.53</td>
</tr>
<tr>
<td>Self-Scratch</td>
<td>25.97</td>
<td>&lt; .01</td>
<td>1.16</td>
<td>.35</td>
<td>9.83</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>Scent Mark</td>
<td>2.30</td>
<td>.19</td>
<td>0.57</td>
<td>.58</td>
<td>0.78</td>
<td>.49</td>
</tr>
</tbody>
</table>

**Figure 2** Interaction between stressor and time for Inactive (nontrained animals).

**Figure 3** Interaction between stressor and time for Self-Scratch (nontrained animals).
rose again at 1600h, with rates similar pre- and poststressor at this time. The inverse of this was seen for self-scratch, with prestressor levels similar throughout all three time periods. However, after the stressor, levels were much higher at 1200h than for the prestressor period. The difference between pre- and poststressor data was reduced at 1400h and virtually was eliminated at 1600h.

When data for trained and nontrained animals were combined, there was significantly less Inactive behavior after the stressor compared with before it. There also was significantly more Locomote, Self-Scratch, Scent Mark after the stressor compared with before it (see Table 4; see Figure 4). Frequencies of Self-Scratch were significantly lower in trained than nontrained animals (see Table 4; see Figure 4). There were significant interactions between training and stressor for Self-Scratch (see Table 4; see Figure 5). Although there was a very slight increase in the amount of Self-Scratch seen in trained animals after the stressor, there was a

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Stressor F(1, 22) p</th>
<th>Training F(1, 10) p</th>
<th>Stressor × Training F(1, 10) p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive</td>
<td>73.82 &lt; .001</td>
<td>0.14 .72</td>
<td>&lt; 0.01 .96</td>
</tr>
<tr>
<td>Locomote</td>
<td>7.08 &lt; .05</td>
<td>0.98 .35</td>
<td>0.72 .42</td>
</tr>
<tr>
<td>Self-Scratch</td>
<td>14.47 &lt; .01</td>
<td>5.17 &lt; .05</td>
<td>6.61 &lt; .05</td>
</tr>
<tr>
<td>Scent Mark</td>
<td>6.24 &lt; .05</td>
<td>1.46 .25</td>
<td>0.05 .83</td>
</tr>
</tbody>
</table>

**FIGURE 4** Mean sample points spent performing each behavior before and after the stressor (for trained and nontrained animals combined) and for trained and nontrained animals (before and after the stressor combined; collapsed across 1200h, 1400h, and 1600h; bars represent standard errors).
large increase in the amount shown by nontrained animals. The prestressor levels of Self-Scratch were similar for both groups; whereas, after the stressor, nontrained animals scratched more than did the trained individuals.

Cortisol Analysis

When prestressor data were compared with data collected on the day of the stressor, there were no significant effects of time or stress on urinary cortisol, $F(2, 18) = 0.92, p = .42$, and $F(1, 9) = 4.45, p = .06$, respectively (see Figure 6).

DISCUSSION

There was a significant reduction in inactivity following administration of the stressor for both trained and nontrained animals. This behavior was the only one that the stressor affected significantly for the trained animals. Possibly, therefore, a decrease in the amount of time spent inactive may be the most sensitive measure of stress for this species.

There was no difference in the time trained animals spent locomoting pre- and poststressor. When data for the two groups were combined, however, there was a significant increase in locomotion poststressor. This is likely to be due to the increased sample size obtained by pooling data from the two groups. These results suggest that, in studies with at least a large sample size, increased levels of locomotion may be a useful and relatively long-lasting measure of stress and possibly reduced welfare. In support of this, Smith, McGreer-Whitworth, and French (1998) found locomotory behavior to be positively correlated with urinary cortisol in Weid’s black, tufted-ear marmosets when housed alone in a novel cage.
There was no significant difference in amount of self-scratching following the stressor in the trained animals. However, the nontrained animals showed a significant increase in self-scratching poststressor. When data for trained and nontrained animals were pooled, there was also an overall significant increase in self-scratching poststressor. The interaction between stress and time for the combined data of trained and nontrained animals indicated that the greatest increase in self-scratching occurred during the earliest observations following the stressor (i.e., at 1200h). Increases in self-scratching, in common with decreases in inactivity, persist for at least 4 hr following stressor administration and were observed at 1400h. Rates of both these behaviors, however, returned to almost prestressor levels by 1600h.

Self-scratching is thought to be a displacement activity in primates, which occurs during situations of tension, anxiety, frustration, conflict, and stress (Maestripieri, Schino, Aureli, & Troisi, 1992). In pharmacological studies, benzodiazepine anxiolytic drugs have been found to reduce the frequency of self-scratching in the common marmoset (Barros, Boere, Huston, & Tomaz, 2000; Cilia & Piper, 1997).

When pre- and poststressor values were combined, the amount of self-scratching was significantly higher in nontrained than trained animals. The positive interaction between training and stress showed that whereas trained animals showed no difference in scratching poststressor, there was an increase in self-scratching in the nontrained animals following the stressor. The fact that amount of self-scratching was similar for both groups prestressor suggests that training animals has no effect on their prestressor, undisturbed behavior. However, evidenced by the similarity between pre- and poststressor levels of self-scratching, being exposed to training procedures may mean that these animals are less affected by stressors than are their nontrained counterparts.
Some researchers consider scent marking to be an anxiety-related behavior in the marmoset, as it is affected by various classes of anxiolytic drugs (Cilia & Piper, 1997). In this study, frequency of scent marking was not significantly different in trained or nontrained animals following the stressor. When data from both groups were pooled, however, there was a significant increase in this behavior poststressor. This suggests that increases in scent marking may be an indicator of stress in this species, albeit less sensitive and requiring a larger sample size to show significance than, for example, self-scratching. There was no significant difference between trained and nontrained animals in the amount of scent marking observed, indicating that training was not a confounding variable on scent-marking behavior.

This study has resulted in a behavioral index of welfare for the common marmoset and has broad implications for the assessment and subsequent improvement of welfare in this species. The measures identified are simple, noninvasive, and easy to implement. They could be used by technicians to assess welfare implications of variations in scientific and husbandry procedures. It should be noted, however, that many behaviors may have wide ranges of acceptable time budgets within which welfare is not compromised. The challenge remains to be able to quantify what frequencies of each behavior are normal and acceptable and at what stage changes in behavioral frequency actually may represent a threat to welfare.

The marmosets in this study showed no significant differences in urinary cortisol levels in relation to the stressor. Possibly, a significant result may have been obtained with a larger sample size. Other studies have found clear increases in urinary cortisol in callitrichid primates in response to a stressor. Isolation in a small cage for approximately 11 hr produced significant increases in urinary cortisol in Weid’s black tufted-ear marmosets (Smith & French, 1997).

The stressor used possibly may not have been aversive enough to provoke a physiological reaction in the trained animals in this study. Supporting this suggestion these animals (the only ones from which urine was collected) showed very little behavioral change following the stressor. Smith and French (1997) used isolation for 11 hr in a novel cage as a stressor; in this study, animals were removed from the homecage for approximately 4 min and, for part of that time, still were in contact with their pair mates. The presence of the familiar pair mate during part of, and following, the stressor also may have attenuated the behavioral and physiological response. The presence of familiar peers has been shown to reduce the impact of stressors in Weid’s black tufted-ear marmosets (Smith et al., 1998). However, it should be noted that even the brief routine stressor used in this study in the presence of a familiar pair mate resulted in behavioral changes associated with stress for an extended period of time. Inactivity and self-scratching did not return to prestressor levels in the nontrained marmosets until 1600h, 6 hr after the stressor.

The increased human contact and interaction that the marmosets underwent because of the training for urine collection also may have had a beneficial effect on
their reactions to being handled and temporarily removed from the homecage. Fear responses in the stressor situation may have been lessened due to the marmosets’ previous experience with human interaction, which mainly was comprised of positive reinforcement and frequent rewards. The trained animals therefore may have perceived the stressor differently than did the nontrained animals, who had less prior experience of positive human interaction. Psychological factors play a significant role in the stress response (Mason, 1968); changed perception of the stressor, therefore, may have altered behavioral and physiological reactions to it. Common marmosets accustomed to handling and bi-weekly cage transfers did not show an immediate elevation in plasma cortisol when exposed to a novel environment with an unfamiliar, opposite-sex partner (Norcross & Newman, 1999). The results of this study, therefore, suggest that exposing marmosets to positive human interaction may help them to cope better with routine laboratory procedures such as being removed from the homecage and weighed.

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