Baseline cortisol levels and social behavior differ as a function of handedness in marmosets (*Callithrix jacchus*)

Emma Vaughan1 | Annie Le1 | Michaela Casey2 | Kathryn P. Workman2 | Agnès Lacreuse2,3

1Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, Massachusetts
2Psychological and Brain Sciences, University of Massachusetts, Amherst, Massachusetts
3Center for Neuroendocrine Studies, University of Massachusetts, Amherst, Massachusetts

Correspondence
Agnès Lacreuse, PhD, Psychological and Brain Sciences, Tobin Hall, 135 Hicks Way, University of Massachusetts, Amherst, MA 01003.
Email: alacreuse@psych.umass.edu

Funding information
NIH, Grant/Award Number: R01AG-046266; William Lee Science Impact fellowship

Abstract
Population hand preferences are rare in nonhuman primates, but individual hand preferences are consistent over a lifetime and considered to reflect an individual’s preference to use a particular hemisphere when engaged in a specific task. Previous findings in marmosets have indicated that left-handed individuals tend to be more fearful than their right-handed counterparts. Based on these findings, we tested the hypotheses that left-handed marmosets are (a) more reactive to a social stressor and (b) are slower than right-handed marmosets in acquiring a reversal learning task. We examined the hand preference of 27 male and female marmosets (ages of 4–7 years old) previously tested in a social separation task and a reversal learning task. Hand preference was determined via a simple reaching task. In the social separation task, monkeys were separated from their partner and the colony for a single 7-hr session. Urinary cortisol levels and behavior were assessed at baseline, during the separation and 24 hr postseparation. Hand preferences were equally distributed between left (n = 10), right-handed (n = 10), and ambidextrous (n = 7) individuals. The separation phase was associated with an increase in cortisol levels and behavioral changes that were similar across handedness groups. However, cortisol levels at baseline were positively correlated with right-handedness, and this relationship was stronger in females than in males. In addition, the occurrence of social behaviors (pre- and postseparation) was positively correlated with right-handedness in both sexes. Baseline cortisol levels did not correlate significantly with social behavior. Acquisition of the reversals was poorer in females than males but did not differ as a function of handedness. We conclude that (a) both stress reactivity and cognitive flexibility are similar across handedness groups and (b) left-handers exhibit less social behavior and have lower basal cortisol levels than ambidextrous and right-handed subjects. The underlying causes for these differences remain to be established.

KEYWORDS
cognition, hand preference, laterality, primate, stress

1 INTRODUCTION

The great majority (about 90%) of humans are right-handed (Annett, 1985; Porac & Coren, 1981), a characteristic already present 2 million years ago according to archeological records (Uomini, 2009). In right-handers, the left hemisphere, which controls language processing, also controls the distal movements of the right hand (Rasmussen & Milner, 1977). Handedness is therefore a behavioral expression of the lateralization of the nervous system (Knecht et al., 2000). Accordingly, we might expect differences between left and
right-handers in selective aspects of cognitive and emotional processing. Equivocal results have been provided regarding cognitive differences between left-handed and right-handed humans. A few studies have reported that left-handers have lower working memory (Powell, Kemp, & García-Finaña, 2012), spatial ability (Somers, Shields, Boks, Kahn, & Sommer, 2015), or spatial and verbal scores (Mellet et al., 2014) than right-handers, while many find no significant difference between the two handedness groups (Corballis, Hattie, & Fletcher, 2008; Peters, Reimers, & Manning, 2006). Other authors have argued that comparing handedness groups should be based on the consistency of hand preferences (consistent vs. inconsistent hand users) rather than the direction (left vs. right) of hand preferences, for example, Prichard, Propper, and Christman (2013). The authors have suggested that inconsistent handedness is associated with increased interhemispheric interaction and access to the right cerebral hemisphere, with better performance of inconsistent hand-users reported in tasks such as episodic memory (Lyle, Hanauer-Torrez, Hacklander, & Edlin, 2012; Propper, Christman, & Phaneuf, 2005).

While evidence for a link between handedness and cognitive ability remains unclear in humans, the data are more consistent with regard to emotional processing. Indeed, several studies have reported that left-handers are more anxious than right-handers (Davidson & Schaffer, 1983; Hicks & Pellegrini, 1978; Merckelbach, de Ruiter, & Olff, 1989; Wright & Hardie, 2012), take longer to start a task, especially if it is novel (Wright & Hardie, 2012; Wright & Hardie, 2015; Wright, Hardie, & Rodway, 2004) and exhibit a greater increase in heart rate than right-handers when confronted to a physical stressor (cold stressor and handgrip dynamometry tests; Jaju, Dikshit, Purandare, & Raje, 2004). These findings are generally consistent with the idea that left-handers are more behaviorally inhibited than right-handers (Wright, Hardie, & Wilson, 2009), a characteristic traditionally associated with right hemisphere processing (Sutton & Davidson, 1997).

While the lateralization of the brain is pervasive among animals and predates the appearance of vertebrates (Bisazza, J. Rogers, & Vallortigara, 1998; Niven & Frasnelli, 2018), handedness (in either direction) at the population level is rare in nonhuman primates and influenced by a variety of factors, such as complexity of the task or posture (Fagot & Vaucclair, 1991; Hopkins, 2006; MacNeiilage, Studdert-Kennedy, & Lindblom, 1987; Warren, 1980). In contrast, hand preferences at the individual level are consistent over the lifetime and have been considered as a marker of an individual’s preference to use a specific hemisphere for a particular task (Rogers, 2018). Indeed, hand preference indicates which hemisphere is most likely to be in use, but does not reflect cerebral lateralization per se, as it does not always correlate with other lateralized behaviors, such as eye preference (Hook-Costigan & Rogers, 1998). Therefore, comparing the performance of left-handed and right-handed individuals in a particular task may provide a window into the cognitive and emotional processes used to perform the task. Only a few studies have compared the cognitive abilities of left-handed and right-handed nonhuman primates. Hörster and Ettlinger (1985) compared the performance of left-handed, right-handed, and ambidextrous rhesus monkeys in the acquisition of a tactile discrimination task. They found that left-handed monkeys acquired the learning criterion faster than right-handed monkeys, but that ambidextrous monkeys were even faster than both groups. Hopkins and Washburn (1994) tested the performance of 26 rhesus monkeys on several computerized tasks including maze-solving tasks, delayed matching to sample tasks and delayed response tasks with a spatial or shape version and examined which hand the subjects used while manipulating the joystick to perform these tasks. They found significant differences between left-handed and right-handed monkeys on the delayed response tasks, in which right-handers performed better on the shape version, while left-handers performed better on the spatial version. Both these studies suggest a left-hand preference/right hemisphere advantage for processing tactual and spatial information. However, not all studies find cognitive differences between left-handed and right-handed monkeys. For example, Cameron and Rogers (1999) tested the abilities of marmosets to slide a door to capture a mealworm from a transparent tube but did not observe any difference between left-handed and right-handed monkeys on several measures of task performance.

The data are more consistent when emotional processing is considered and remarkably in line with the human findings (reviewed in Wright & Hardie, 2015) in suggesting that left-handers have more trait anxiety and behavioral inhibition than right-handers (Rogers, 2018). In one study, Gordon and Rogers (2015) examined the cognitive bias of marmosets in response to ambiguous cues that could be interpreted as positive or negative. They trained marmosets to associate a white cue with a positive reward and a black cue with a negative experience (lack of reward). Half the marmosets were trained on the opposite contingencies. Subsequently, the experimenters presented the subjects with a gray cue. Interestingly, right-handed marmosets were more likely to treat the gray cues as positive, whereas the left-handed marmosets tended to respond as if the gray cues were negative. In another study, Braccini and Caine (2009) explored marmosets’ reactivity towards novel foods. The authors concluded that marmosets who preferentially reached for food with their right hand were significantly more likely to explore novel situations than left-handed marmosets, suggesting that left-handed marmosets were more cautious when confronted to novelty. Also consistent with these data, Cameron and Rogers (1999) noted that left-handers were less likely to explore the apparatus in the mealworm tube task and Rogers (1999) reported that left-handed marmosets were more fearful than right-handed monkeys when presented with a predatory stressor. In addition, Rogers (2018) indicated that left-handed marmosets show more prolonged elevation of cortisol than right-handed subjects after a day spent in an unfamiliar environment, supporting the hypothesis that left-handed marmosets may have an overactive hypothalamus–pituitary–adrenals (HPA) axis compared with their right-handed counterparts. However, the relationship between cortisol and handedness is unclear, as contradictory data have been reported in rhesus monkeys, with cortisol levels following capture correlating positively.
with the strength of the right-hand preference later in life (Westergaard, Lussier, Suomi, & Higley, 2001).

Overall, reports of cognitive differences between left-handed and right-handed monkeys are scarce but suggest that left-hand preference may confer an advantage on some spatial and tactile tasks. There is far more evidence in support of the hypothesis that left-hand preference is associated with increased cautiousness in novel situations, behavioral inhibition, fearfulness, and a negative cognitive bias. However, the potential relationships between such a behavioral profile and cortisol levels are not clear.

In the present study, we examined how handedness may affect cognitive performance and responses to a social stressor in adult marmosets of both sexes. Based on the literature reviewed above, we hypothesized that left-handedness would be associated with increased fearfulness/overactive HPA axis and predicted that left-handed marmosets would be more reactive than right-handed marmosets to the social stressor. We also predicted that, based on cautiousness with regard to novel situations, left-handers would be slower than right-handed monkeys in acquiring a task of cognitive flexibility in a computerized setting.

2 | MATERIALS AND METHODS

2.1 | Subjects

A total of 27 adult common marmosets (*Callithrix jacchus*), between the ages of 4–7 years (Table 1) participated in this project (14 females and 13 vasectomized males). They were housed in heterosexual pairs in steel mesh cages (101 × 76.2 × 78.7 cm) equipped with perches, hammock, nest boxes and branches to encourage species-typical behaviors. They were maintained at an ambient temperature of 80°F with a relative humidity of 50%. The monkeys were provided with daily enrichment, including foraging tubes and a variety of toys. The research was consistent with the American Society of Primatologists Principles of Ethical Treatment of Non-Human Primates. The animals were cared for in accordance with the guidelines of the US National Research Council’s Guide for the Care and Use of Laboratory Animals, the US Public Health Service’s Policy on Humane Care and Use of Laboratory Animals, and the Guide for the Care and Use of Laboratory Animals (2011), 8th edition. The studies were approved by the University of Massachusetts Institutional Animal Care and Use Committee.

2.2 | Tasks

2.2.1 | Simple reaching task

Hand preference assessments occurred within a month of monkeys completing their first year of cognitive testing on CANTAB and within 6 months of the social separation task. Hand preference was determined from a simple reaching task in which monkeys had to reach with one hand for a small reward (mini-dried marshmallow, diameter 0.63 cm) placed in the middle of the Valley Staircase apparatus of the Hill and Valley test (see Figure 1). The Valley apparatus consists of two staircases rising away from a central vertical slot. The mini-marshmallow was placed across the central slot, in the middle of each staircase. This apparatus was selected before monkeys performing the Hill and Valley task for another study (reported in Workman, Healey, Carlotto, & Lacreuse, 2019).

A left (L) or right (R) score was recorded if the subject used one hand to reach for the reward. Any trials in which the marmoset used both hands were excluded. Each subject performed a maximum of 20 trials each session and completed a total of 50 trials. For each subject, a handedness index (HI) was determined by subtracting the number of right-handed responses from the number of left-handed responses and dividing by the total number of responses (Hopkins, 1999). HI values ranged from −1.0 to 1.0, with the absolute value representing the strength of the preference. The positive values indicated a right-hand bias while the negative values indicated a left-hand bias. The absolute value of [HI] represented the strength of the preference independent of the direction of the preference. In addition, subjects were classified as left-, ambidextrous, or

### TABLE 1 Marmoset characteristics, hand preference, handedness index (HI), and z score

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Hand preference</th>
<th>HI</th>
<th>z score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.96</td>
<td>Female</td>
<td>R</td>
<td>0.72</td>
<td>5.09</td>
</tr>
<tr>
<td>2</td>
<td>4.01</td>
<td>Female</td>
<td>R</td>
<td>0.48</td>
<td>3.39</td>
</tr>
<tr>
<td>3</td>
<td>4.34</td>
<td>Female</td>
<td>R</td>
<td>0.48</td>
<td>3.39</td>
</tr>
<tr>
<td>4</td>
<td>5.01</td>
<td>Female</td>
<td>R</td>
<td>0.48</td>
<td>3.39</td>
</tr>
<tr>
<td>5</td>
<td>5.21</td>
<td>Female</td>
<td>R</td>
<td>0.28</td>
<td>1.98</td>
</tr>
<tr>
<td>6</td>
<td>5.59</td>
<td>Female</td>
<td>R</td>
<td>0.76</td>
<td>5.37</td>
</tr>
<tr>
<td>7</td>
<td>4.34</td>
<td>Female</td>
<td>L</td>
<td>−0.68</td>
<td>−4.81</td>
</tr>
<tr>
<td>8</td>
<td>4.36</td>
<td>Female</td>
<td>L</td>
<td>−0.4</td>
<td>−2.83</td>
</tr>
<tr>
<td>9</td>
<td>4.67</td>
<td>Female</td>
<td>L</td>
<td>−0.28</td>
<td>−1.98</td>
</tr>
<tr>
<td>10</td>
<td>4.71</td>
<td>Female</td>
<td>L</td>
<td>−0.48</td>
<td>−3.39</td>
</tr>
<tr>
<td>11</td>
<td>5.52</td>
<td>Female</td>
<td>L</td>
<td>−0.56</td>
<td>−3.96</td>
</tr>
<tr>
<td>12</td>
<td>6.16</td>
<td>Female</td>
<td>L</td>
<td>−0.72</td>
<td>−5.09</td>
</tr>
<tr>
<td>13</td>
<td>4.25</td>
<td>Female</td>
<td>Ambi</td>
<td>0.12</td>
<td>0.85</td>
</tr>
<tr>
<td>14</td>
<td>4.81</td>
<td>Female</td>
<td>Ambi</td>
<td>−0.16</td>
<td>−1.13</td>
</tr>
<tr>
<td>15</td>
<td>3.92</td>
<td>Male</td>
<td>R</td>
<td>0.56</td>
<td>3.96</td>
</tr>
<tr>
<td>16</td>
<td>4.93</td>
<td>Male</td>
<td>R</td>
<td>0.48</td>
<td>3.39</td>
</tr>
<tr>
<td>17</td>
<td>5.04</td>
<td>Male</td>
<td>R</td>
<td>0.48</td>
<td>3.39</td>
</tr>
<tr>
<td>18</td>
<td>6.51</td>
<td>Male</td>
<td>R</td>
<td>0.92</td>
<td>6.50</td>
</tr>
<tr>
<td>19</td>
<td>4.64</td>
<td>Male</td>
<td>L</td>
<td>−0.72</td>
<td>−5.09</td>
</tr>
<tr>
<td>20</td>
<td>4.81</td>
<td>Male</td>
<td>L</td>
<td>−0.48</td>
<td>−3.39</td>
</tr>
<tr>
<td>21</td>
<td>4.86</td>
<td>Male</td>
<td>L</td>
<td>−0.32</td>
<td>−2.26</td>
</tr>
<tr>
<td>22</td>
<td>5.57</td>
<td>Male</td>
<td>L</td>
<td>−0.32</td>
<td>−2.26</td>
</tr>
<tr>
<td>23</td>
<td>4.87</td>
<td>Male</td>
<td>Ambi</td>
<td>0.12</td>
<td>0.85</td>
</tr>
<tr>
<td>24</td>
<td>4.88</td>
<td>Male</td>
<td>Ambi</td>
<td>0.08</td>
<td>0.56</td>
</tr>
<tr>
<td>25</td>
<td>5.04</td>
<td>Male</td>
<td>Ambi</td>
<td>−0.08</td>
<td>−0.56</td>
</tr>
<tr>
<td>26</td>
<td>5.39</td>
<td>Male</td>
<td>Ambi</td>
<td>−0.08</td>
<td>−0.56</td>
</tr>
<tr>
<td>27</td>
<td>5.73</td>
<td>Male</td>
<td>Ambi</td>
<td>0.2</td>
<td>1.41</td>
</tr>
</tbody>
</table>
FIGURE 1  Marmoset performing the simple reaching hand preference test in the Valley apparatus

right-handed based on binomial z scores calculated from the frequency of left- and right-hand responses. Subjects with z scores of −1.64 or lower were classified as left-handed and those with z scores of 1.64 or higher were classified as right-handed. All others were classified as ambidextrous.

2.2.2 | Temporary social separation and cortisol assays

Following the procedure of French et al. (2012), each marmoset was removed from its colony for 7 hr and placed alone in an unfamiliar room, in a cage similar to their home cage. The monkeys could not see, smell or hear conspecifics during the separation phase. Food and water was provided ad libitum. Behavior was video-recorded with a SONY Handycam (HDD 2000x digital zoom) video camera provided with 0.45x wide angle lens. Animals were video-recorded at Baseline (30 min before separation), throughout the separation period, upon reunion with partner, and 24 hr postseparation (the day following separation). Behaviors included locomotor, social, and anxiety measures adapted from an extensive ethogram developed for the marmoset Stevenson and Poole (1976), as well as vocalizations. Behavior was scored from the video recordings using an interval schedule of 15 s for 5 min. All experimenters achieved 90% interrater reliability on behavioral observations before scoring videos.

Urinary samples were collected at Baseline, every hour during the separation phase and 24 hr postseparation. Urine samples were analyzed for cortisol at the Endocrine BioServices Assay Laboratory of the University of Nebraska at Omaha, using an in-house assay kit. The intra-assay coefficient of variation was 4.32%, the interassay C.V. was 16.6%. Because few monkeys provided urine within the first 2 hr of separation, cortisol values were averaged for baseline (15 min before separation), middle separation (3–5 hours), end of the separation (last 2 hours), and postseparation (24 hr postseparation).

The stress reactivity data of a subset of animals have been reported in LaClair et al. (2019).

2.2.3 | Cognitive performance

The cognitive data were collected throughout 2016, with data from a subset of monkeys presented in several papers (LaClair et al. 2019; Lacreuse, Moore, LaClair, Payne, & King, 2018; Workman et al., 2019). For most monkeys, cognitive performance was assessed in a reversal learning task administered on a touchscreen (CANTAB battery, see Spinelli et al., 2004) and described in details in Workman et al. (2019). Briefly, the monkeys were presented sequentially with three pairs of stimuli. For each pair, they had to perform a simple discrimination (SD) to determine which of the two stimuli was rewarded until they reached a 90% correct learning criterion, after which a simple reversal (SR) was given. For SR, the stimulus/reward contingencies were reversed and the monkey had to select the other stimulus of the pair to be rewarded. Monkeys performed a SD and SR for each of the three pairs of stimuli (SD1 though SR3) and performed a total of 40 trials a day on each test. The stimuli appeared at random positions on the screen. Five monkeys (3 females and 2 males) were unable to learn to perform tasks on touchscreen and were trained on a manual version of the tests (see Workman et al., 2019 for a detailed description). These monkeys were tested in a Wisconsin General Testing Apparatus (WGTA)-like box which contained a test tray with two food wells. The wells could be baited with mini-dried marshmallows and covered by stimulus objects. These stimuli were made of foamy material of the same shape and colors as the stimuli used for the touchscreen version. The procedure was similar as for the touchscreen, except that trials were administered by an experimenter, only 20 trials per day were given to minimize satiety effects, and the correct stimulus was randomly placed at the left or right side of the tray. Between trials, the tray was concealed from view by an opaque screen. The trials to reach criterion (TTC) was calculated for each of the six tests. To summarize performance, a reversal index RI was computed for each monkey by dividing the averaged TTC across the three reversals by the averaged TTC for the three discriminations.

2.2.4 | Statistical analysis

Independent Student’s t tests were used to examine the effect of Sex on HI and |HI|. Analyses of variance (ANOVAs) with Sex and Hand Preference (Left, Right, and Ambidextrous) as factors were used to examine differences in age, weight, and cognitive performance (RI) as a function of Hand Preference in males and females. Because cortisol data were not available for all time points in all subjects (i.e., monkey did not urinate), linear mixed models were used to examine the effects of Sex, Phase (Baseline, Middle, End, and Postseparation) and Hand Preference on cortisol levels. Pearson’s correlations were used to examine the relationships between HI and cortisol.

For the behavioral data, we analyzed one locomotor measure indicative of a relaxed state (Calm Locomotion), one locomotor
measure reflecting a stressed state (Agitated Locomotion), Inactive Alert, indicating heightened vigilance, as well as Inactive Rest, when the animal is calmly resting. Social behaviors (social play, social contact, mounting, grooming, sniffing, and tongue flick) were summed into a single Social category. By definition, social behaviors were not observed during the separation, as the focal animal was alone during this phase. Social behaviors observed at Baseline and 24 hr later were not significantly different and were averaged for the analysis. Vocalizations were scored during the separation only, as it was not possible to determine whether vocalization heard during the Baseline and postseparation phase (i.e., when other marmosets were present) were produced by the focal animal or other animals in the colony. However, too few vocalizations were produced for a meaningful analysis. Because the behavioral data were not normally distributed, the Friedman test for related samples, followed by a post hoc the Wilcoxon signed rank test, was used to assess the effect of Phase on these measures. The Kruskal-Wallis test, followed by a Dunn’s test with Bonferroni correction, was used to assess differences among handedness groups. Spearman Rho was used to examine correlations between HI, basal cortisol and the behavioral measures. A linear regression was performed to examine the relationships between basal cortisol, HI, Sex, and RI.

3 | RESULTS

The HI, z score, and hand preference for each monkey in the simple reaching task can be seen in Table 1. The number of left (n = 10), right (n = 10), and ambidextrous monkeys (n = 7) did not differ significantly. Sex did not affect the direction (t(25) = 0.32, p = .75) or the strength (t(25) = −1.09, p = .29) of hand preferences. The weights were similar across Sex (F(1, 21) = 0.66, p = .42), Handedness (F(2, 21) = 0.67, p = .41), and Sex × Handedness (F(2, 21) = 0.73, p = .49) categories. Similarly, the ages did not differ as a function of Handedness (F(2, 21) = 0.53, p = .94), Sex (F(1, 21) = 1.65, p = .21), or their interaction (F(2, 21) = 0.45, p = .64).

A linear mixed model was used to analyze cortisol levels as a function of Phase of the separation task, Sex, and Hand Preference. Cortisol levels differed significantly according to Phase F(3, 15.41) = 14.34, p = .001), reflecting a significant cortisol increase from Baseline for each time point. Contrary to our hypothesis, Hand Preference did not affect cortisol levels (F(2, 15.18) = 0.032, p = .73) at any time point during the social separation task (Phase × Hand Preference interaction; F(6, 19.33) = 1.07, p = .42; Figure 2). Sex (F(1,14.89) = 1.62, p = .22) and its interaction with Hand Preference (F(2, 15.56) = 0.48, p = .63) did not significantly affect cortisol levels. However, a marginal three way interaction Phase × Hand Preference × Sex (5, 18.14) = 2.47, p = .071) indicated complex associations between Sex, Hand Preference, and separation Phase. Follow-up analyses were conducted separately for each phase to explore this effect further.

The analyses revealed that cortisol levels at baseline tended to differ according to Hand Preference (F(2, 21) = 2.67, p = .092). In addition, the interaction between Hand Preference and phase in the social separation test. *p < .05

![Figure 2](image)

**FIGURE 2** Urinary cortisol levels as a function of hand preference and phase in the social separation test. *p < .05

![Figure 3](image)

**FIGURE 3** Baseline urinary cortisol levels as a function of hand preference in male and female marmosets. *p < .05
The behavioral data were first analyzed according to the effect of Phase. Whereas Calm Locomotion and Inactive Rest did not differ among the three phases, Agitated Locomotion ($\chi^2(2) = 28.29$, $p = .001$) was significantly greater during the separation than during the Baseline ($Z = -3.20$, $p = .001$) and Postseparation ($Z = -3.87$, $p = .001$) Phases. Similarly, Inactive Alert ($\chi^2(2) = 16.77$, $p = .001$) was significantly greater during the separation than during the Baseline ($Z = -3.07$, $p = .002$) and Postseparation Phases ($Z = -3.09$, $p = .002$). Thus, the social separation procedure induced the intended temporary stress, with quickly returned to baseline levels 24 hr later.

Differences associated with Hand Preference were analyzed with the Kruskal–Wallis test. Because the Baseline and Post conditions did not differ significantly for any of the variables considered (paired t tests, all $p > .5$), the behavioral measures within each category (Social, Calm Locomotion, Agitated Locomotion, Inactive Alert, and Inactive Rest) were averaged between these two conditions. Interestingly, only Social behaviors differed significantly according to Hand Preference ($H(2, 27) = 9.30$, $p = .01$). Post hoc tests revealed that left-handers exhibited significantly fewer social behaviors than both right-handers ($p = .029$) and ambidextrous marmosets ($p = .027$). To examine whether this effect was present in both sexes, the same analysis was run in females and males separately. The results indicated that left-handers exhibited fewer social behaviors than the two other groups in both males ($H(2, 13) = 7.74$, $p = .021$) and females (after removal of one outlier, $H(2, 13) = 6.65$, $p = .036$; Figure 5). In addition, HI was positively correlated with social behaviors ($\rho = 0.59$, $p = .002$), a relationship significant in both males ($\rho = 0.67$, $p = .013$) and females ($\rho = 0.59$, $p = .031$; Figure 6). Importantly, basal cortisol levels were not significantly correlated with social behaviors ($\rho = 0.11$, $p = .58$).

For cognitive performance, a main effect of Sex ($F(1, 21) = 10.90$, $p = .003$) on RI indicated that females were slower than males in acquiring the reversals, compared to the simple discriminations, a finding reported elsewhere (LaClair et al., 2019; Lacreuse et al., 2018; Workman et al., 2019). However, neither Hand Preference ($F(2, 21) = 0.14$, $p = .87$) nor the interaction between Sex and Hand Preference ($F(2, 21) = 0.017$, $p = .98$; see Figure 7) significantly affected RI. The results were not significantly changed when age or weight were included as covariates in the analysis.

Finally, a linear regression was performed to examine potential predictors of cognitive performance. A model including basal cortisol, HI, and Sex as variables explained 40% of the variance in cognitive performance ($F(3, 23) = 6.77$, $p = .002$). Sex ($\beta = 0.50$, $t = -3.14$, $p = .005$) was a significant predictor of RI, with the same trend for basal cortisol ($\beta = 0.35$, $t = 2.04$, $p = .053$), while HI was not a significant predictor ($\beta = -0.9$, $t = -0.52$, $p = .60$).

**FIGURE 4** Positive correlation (Pearson’s $r = 0.38$, $p = .05$) between basal cortisol levels and Handedness Index (HI) in males and female marmosets. The correlation is significant in females ($r = 0.72$, $p = .004$), but not in males ($r = -.22$, $p = .46$).

**FIGURE 5** Occurrence of social behaviors (mean baseline and 24hr-postseparation) as a function of hand preference in male and female marmosets. *$p < .05$.

**FIGURE 6** Occurrence of social behaviors (mean baseline and 24hr-postseparation) as a function of hand preference in male and female marmosets. *$p < .05$.

**DISCUSSION**

Hand preference is a manifestation of an individual’s preference to use a particular hemisphere, providing a window into the cognitive and emotional processes characteristic of the individual when performing the task (Rogers, 2018). Our measurements of hand preference in marmosets using a simple reaching task confirm that most marmosets have an individual hand preference, but that left- and right-handers are equally represented at the population level.

Our study focused on the effect of hand preference on cognitive flexibility and reactivity to a social stressor. With regard to cognitive flexibility, as assessed by reversal learning, we found that performance was equivalent between left-, right-handers and ambidextrous marmosets. Independent of hand preference, however, females obtained poorer performance than males, a finding that we have reported previously (LaClair et al., 2019; Lacreuse et al., 2018; Workman et al., 2019). The lack of handedness influence on cognitive performance is consistent with several reports in humans (e.g., Corballis et al., 2008) and nonhuman primates including marmosets (e.g., Cameron & Rogers, 1999).

Prior studies have linked hand preference to various exploratory and social behaviors (Braccini & Caine, 2009; Gordon & Rogers, 2015). Left-handed marmosets have been found to react more negatively to novel or an ambiguous situation (Braccini & Caine,
Braccini and Caine (2009) demonstrated that left-handed marmosets have a greater fear response towards a predatory call. Based on these data, we predicted that left-handed marmosets would be more reactive to a social stressor than right-handed marmosets. Contrary to this prediction, cortisol levels during or after the social separation did not differ significantly according to hand preference. The discrepancy with previous research may stem from the type of stressor used. As noted above, Gordon and Rogers (2015) measured reactivity of the monkeys in face of a novel task, while Braccini and Caine (2009) measured responses to a fearful situation. Additional evidence that fearful situations induce right hemisphere activation in marmosets comes from a recent study using tympanic membrane temperature thermometry (Pereira, Duarte, Maior, & Barros, 2018). The authors found that changes in temperature after exposure to two types of threatening stimuli (human intruder or predator) were specific to the right ear (i.e., right hemisphere) in marmosets. It is likely that the temporary social separation procedure that we used in our study was anxiety rather than fear inducing. Accordingly, it is possible that left and right-handers express differential responses in the face of threatening or novel stimuli, but not in the context of mild anxiety.

While cortisol responses to the social separation were similar between handedness groups, significant differences emerged in baseline cortisol levels, that is, in urine samples collected before the separation. Contrary to our predictions, right-handed female marmosets had significantly higher baseline cortisol levels than all other groups. In addition, the right-hand bias in females was highly correlated with basal cortisol levels, strengthening the validity of this finding. Interestingly, Westergaard et al. (2001) found similar results in female rhesus monkeys and reported plasma cortisol levels (sampled when monkeys were juvenile) to be positively related to right-hand usage as adults. The mechanisms underlying an association between handedness, basal cortisol, and sex are unknown. However, a recent study in male marmosets reported that higher hair cortisol correlated positively with sociability (Inoue-Murayama, Yokoyama, Yamanashi, & Weiss, 2018). Although we did not evaluate personality in this study, we measured the occurrence of social behaviors with the cage partner pre- and 24 hr postseparation. We found that the occurrence of social behaviors was lowest in left-handers and positively correlated with right-handedness. However, no correlation was found between basal cortisol and social behaviors. Unfortunately, we were not able to analyze vocalizations, which may provide a critical link between lateralization and social behavior. Indeed, hand preference has long been shown to be associated with the lateralization of language in humans (Mazoyer et al., 2014; Rasmussen & Milner, 1977), with the great majority of right-handed people having language represented in their left hemisphere. Interestingly, in marmosets, the production of social contact vocalizations (i.e., twitter calls) is also associated with a greater involvement of the left hemisphere (Hook-Costigan & Rogers, 1998). It is therefore plausible that highly social individuals develop right-hand bias associated with the production of social signals. This hypothesis will
have to be tested in future studies recording the frequency and type of vocalizations produced in different social contexts.

Right-handed individuals (especially females) were found to have elevated cortisol and the levels correlated positively with right-handedness. This finding could reflect enhanced sociability, based on the study of Inoue-Murayama et al. (2018) which reported a positive correlation between hair cortisol and sociability in male marmosets. Alternatively, elevated basal cortisol could reflect stress exposure or metabolic dysfunction. Two sets of findings argue against such a hypothesis. First, we found no evidence that stress reactivity differs as a function of handedness, and second, the association between cortisol and HI remained significant after controlling for weight or age, making it unlikely that compromised metabolic function due to obesity or aging underlined the relationship between cortisol and handedness.

An alternative hypothesis is that both handedness and basal cortisol levels may be related to social rank. In the study by Inoue-Murayama et al. (2018), hair cortisol was not significantly associated with dominance after controlling for other variables, but the study was conducted in males only. In female marmosets, Saltzman, Schultz-Darken, Wegner, Wittwer, and Abbott (1998) reported that dominant individuals show higher plasma cortisol levels compared to subordinate individuals. Therefore in our study, higher (urinary) cortisol at baseline could represent females with dominant status. Although only few studies have directly examined the relationships between social rank and handedness, they have provided little evidence for such an association. Of particular interest, one study conducted in another New World species, the squirrel monkey, reported that social rank did not significantly influence preferential hand use (Laska, 1996). However dominant status is often associated with reproductive function (cycling vs. anovulatory status) in female marmosets (Saltzman, Schultz-Darken, Scheffler, Wegner & Abbott, 1994). Even though our monkeys were housed in heterosexual pairs, as opposed to social groups, it is possible that some females experienced reproductive suppression and low circulating cortisol levels (Saltzman et al., 1998). Thus, examining social rank as a potential link between cortisol levels and handedness is warranted.

Finally, marmosets with higher basal cortisol tended to perform worse on the cognitive task. Negative associations between basal cortisol and cognitive performance have been found in several human studies (Comijs et al., 2010; Kalmijn et al., 1998; Lee et al., 2007; Lupien et al., 1994) and are often interpreted in the context of a dysregulated HPA axis. Additional studies are needed to better understand whether higher basal cortisol in marmosets represent HPA axis dysregulation, dominant status or metabolic alterations that affect cognitive performance.

5 | CONCLUSION

This study in middle-aged common marmosets examining the relationships between hand preference, cortisol levels and cognitive performance in both sexes revealed several intriguing results. Contrary to our hypotheses, we found no evidence that left-handed monkeys are more reactive to a social stressor or slower in acquiring a cognitive task than right-handed monkeys. In contrast, an unexpected association between right-handedness and greater basal cortisol levels (in females) and greater social behavior (in both sexes) was found. We suggest that right-hand preference in highly social individuals may reflect a left hemisphere dominance for the production of social contact vocalizations. The relationship between elevated cortisol and right-handedness is more difficult to interpret, but may be related to some aspects of personality, dominant status and/or metabolism not captured by the present study. As basal cortisol levels tend to predict cognitive flexibility, it will be important for future studies to determine the factors that contribute to elevated basal cortisol in marmosets.

ACKNOWLEDGMENTS

This study was supported by NIH grant R01AG-046266 to A. Lacreuse. Michaela Casey was supported by the William Lee Science Impact Fellowship. We are particularly grateful to all the students who participated in data collection and to the UMass Psychology shop and Animal Care staff for their excellent assistance.

DATA AVAILABILITY STATEMENT

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

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ORCID

Agnès Lacreuse http://orcid.org/0000-0003-2890-9649

REFERENCES


---

**How to cite this article**: Vaughan E, Le A, Casey M, Workman KP, Lacreuse A. Baseline cortisol levels and social behavior differ as a function of handedness in marmosets (Callithrix jacchus). *Am J Primatol.* 2019;81:e23057. https://doi.org/10.1002/ajp.23057