A study of lateralized behaviours in domestic horses (Equus caballus)

Ashley Crosby

Examinator, Carlos Guerrero Bosagna
Tutor, Matthias Laska
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**Författare**
Ashley Crosby

**Sammanfattning**
Lateralized behaviour is the most conspicuous manifestation of hemispheric specialization of the brain and has been reported in a variety of taxa. Only a few studies have so far assessed lateralized behaviours in horses. Therefore, I observed ten domestic horses for 16 weeks for an array of spontaneously occurring motor behaviours as well as stimulus-induced behavioural responses to determine if they display side preferences at the individual or population level and to assess possible correlations between lateralized behaviours. Significant side preferences were found for certain behaviours at the individual level, ranging from standing and flexing, to auditory stimuli, and olfactory stimuli. All horses showed task-dependent changes in their side preferences and no significant side preferences were found at the population level for any behaviours. Similarly, no significant correlations were found between behaviours. Taken together, the results of the present study suggest that horses, like all other species studied so far except humans and some great apes, only display lateralized behaviour at the individual, but not at the population level.

**Nyckelord**
Lateralization, domestic horse (*Equus caballus*), motor/social/sensory behaviours, individual-level lateralization, population-level lateralization
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1. Abstract

Lateralized behaviour is the most conspicuous manifestation of hemispheric specialization of the brain and has been reported in a variety of taxa. Only a few studies have so far assessed lateralized behaviours in horses. Therefore, I observed ten domestic horses for 16 weeks for an array of spontaneously occurring motor behaviours as well as stimulus-induced behavioural responses to determine if they display side preferences at the individual or population level and to assess possible correlations between lateralized behaviours. Significant side preferences were found for certain behaviours at the individual level, ranging from standing and flexing, to auditory stimuli, and olfactory stimuli. All horses showed task-dependent changes in their side preferences and no significant side preferences were found at the population level for any behaviours. Similarly, no significant correlations were found between behaviours. Taken together, the results of the present study suggest that horses, like all other species studied so far except humans and some great apes, only display lateralized behaviour at the individual, but not at the population level.

Keywords: lateralization, domestic horse (Equis caballus), motor/social/sensory behaviours, individual-level lateralization, population-level lateralization

2. Introduction

Hemispheric specialization has been reported in a variety of taxa, including mammals, birds, fish, and insects and is a widespread phenomenon among vertebrates (Farmer et al., 2018). In mammals, it is thought that the left hemisphere is used to control routine, i.e. day-to-day responses, that have been established through learning whereas the right hemisphere is used in emergency situations, social interaction and expression of aggression and fear (Austin and Rogers 2012).

Laterality in any species can be manifested as morphological, sensory, or functional asymmetry (Murphy and Arkins, 2006). Lateralized behaviour, which can be demonstrated as sensory or functional laterality, is the most conspicuous expression of hemispheric specialization (Basile et al., 2009). Lateralized behaviour has been reported to occur at two levels: 1) individual-level lateralization i.e. lateralization in a single individual regardless of a common direction bias in the population, and 2) population-level lateralization i.e. lateralization in a number of individuals, representing a population bias when 60-90% of individuals demonstrate the same direction of lateralization (Frasnelli and Vallortigara, 2018). Individual level laterality has been
reported to occur in a variety of species. (Austin and Rogers, 2007; Leliveld, 2019). Whereas lateralized behaviour at the population level – such as human right-handedness – is scarce. (Gotts et al., 2013). Nevertheless, certain lateralized behaviours observed in a given species have been reported to correlate with each other, for example handedness and footedness in humans (Gotts et al., 2013).

Studies on lateralized behaviour have so far mainly focused on nonhuman primates – due to their close phylogenetic relationship with humans – whereas studies on nonprimate species such as ungulates have been more limited. A review article on laterality research completed on ungulate livestock by Leliveld (2019) established that in the last two decades, the number of studies on laterality of ungulates, including cattle, sheep, goat, pig, donkey, buffalo and horses has increased; prior to that, the number of articles was very low (Leliveld, 2019). The majority of studies have so far focused on motor laterality, however, studies are increasingly focusing on other lateralized functions, such as cognition and emotion (Leliveld, 2019). Studies have also assessed the impact of age, sex, personality, health and stress on laterality (Leliveld, 2019).

Among the ungulates, studies on horses have shown the largest increase in the number of peer-reviewed journal articles published (in English) per decade. They are a particularly suitable species for studying lateralized behaviours due to their laterally placed eyes, separate nostrils, individually movable ears and their tendency to display side-biases in certain behaviours (Inoue et al., 2020; Austin and Rogers, 2012; Leliveld, 2019).

Lateralization in horses has been studied in a variety of motor behaviours, such as placing one foreleg in front of the other while standing or grazing (Austin and Rogers, 2012; Byström et al., 2020; Esch et al., 2019; van Heel et al., 2006; Wells and Blanche, 2008), flexing one foot while standing or grazing (McGreevy and Rogers, 2005; McGreevy and Thompson, 2006), leading limb when moving (Marr et al., 2018; Murphy et al., 2005; Warren-Smith and McGreevy, 2010), autogrooming (McGreevy and Rogers, 2005), rubbing (McGreevy and Rogers, 2005), rolling (Murphy and Arkins, 2006), pawing (McGreevy and Rogers, 2005), lying down (McGreevy and Rogers, 2005), competition i.e. racing and dressage (Cully et al., 2018; Deuel and Lawrence, 1987; Whishaw 2015; Whishaw and Kolb, 2017; Williams and Norris 2007), and trot derailment (Lucidi et al., 2013). Several social behaviours have also been studied for the occurrence of lateral biases, including affiliative interaction (Farmer et al., 2018), agonistic interaction (Austin and Rogers, 2012), and mother and foal interaction (Karenina et al., 2018; Komarkova and Bartsova, 2013; van Dierendonck et al., 2005). In addition, several sensory behaviours have been studied in horses for the occurrence of lateral
biases, including vigilance and reactivity (Austin and Rogers, 2012), as well as responses to auditory stimuli (Basile et al., 2009), olfactory stimuli (Larose et al., 2006; McGreevy and Rogers, 2005; Siniscalchi et al., 2015) and visual stimuli (De Boyer Des Roches et al., 2008). Most of the studies mentioned above found lateralized behaviour at the individual level, but none at the population level (Leliveld, 2019).

With all of the studies so far conducted, none has assessed more than one or two behaviours at the same time in a given study population. Accordingly, only one study by McGreevy and Rogers (2005) assessed a possible correlation between the foot preference when standing or grazing with the first nostril used to smell, and total number of inhalations. However, no significant correlation was found between the motor and sensory behaviours compared in that study. Due to the lack of studies that assess a wide range of potentially lateralized behaviours in a given study population, it is currently not known whether certain lateralized behaviours observed in horses may correlate with each other or occur at the population level.

It was therefore the aim of the present study to 1) determine if lateralized behaviour is present in a catalogue of natural behaviours in individual horses, 2) determine if horses display lateralized behaviour at the population level, and 3) provide the first comprehensive analysis of correlation of lateralized behaviours in horses.

3. Materials and methods

3.1 Animals and location

Ten domestic horses (Equus caballus) maintained at Churchill Chimes Equestrian Centre located in Toronto, Ontario, Canada, were studied. All horses lived outside 24/7 all year round. The horses were kept in a grass pasture during the night (Figure 1A) and brought into a dirt paddock during the day from about 9am-8pm (Figure 1B). The horses always had access to water, were allowed to graze ad libitum in the grass pasture and had access to hay in the dirt paddock. They were brought into the barn around 9am for 20 minutes and fed a mixture of sweet feed, flax and mash (Fit+Fiber and Phase 5) depending on calculated nutritional needs.

Figure 1: A) Grass pasture and B) dirt paddock.
One intact mare and nine geldings of the same herd were chosen. All were in good health, i.e. without lameness or acute illness. They were trained in the English riding style, meaning interactions between horse and human are mainly on the left side i.e. approaching, leading horse, mounting and dismounting, and all horses were ridden by multiple riders.

Table 1: Characteristics of ten studied domestic horses.

<table>
<thead>
<tr>
<th>Horses</th>
<th>Breed</th>
<th>Age (years)</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norton</td>
<td>Canadian</td>
<td>13</td>
<td>Gelding</td>
</tr>
<tr>
<td>Guinness</td>
<td>Clyde cross</td>
<td>19</td>
<td>Gelding</td>
</tr>
<tr>
<td>James</td>
<td>Thoroughbred</td>
<td>29</td>
<td>Gelding</td>
</tr>
<tr>
<td>SunDance</td>
<td>Arab</td>
<td>30</td>
<td>Gelding</td>
</tr>
<tr>
<td>Loki</td>
<td>Welsh cross</td>
<td>22</td>
<td>Gelding</td>
</tr>
<tr>
<td>Munchy</td>
<td>Quarter horse percheron cross</td>
<td>26</td>
<td>Gelding</td>
</tr>
<tr>
<td>Mickey</td>
<td>Quarter horse</td>
<td>21</td>
<td>Gelding</td>
</tr>
<tr>
<td>Sunny</td>
<td>Appaloosa</td>
<td>14</td>
<td>Gelding</td>
</tr>
<tr>
<td>JellyBean</td>
<td>Quarter horse cross</td>
<td>12</td>
<td>Mare</td>
</tr>
<tr>
<td>Rolo</td>
<td>Canadian</td>
<td>15</td>
<td>Gelding</td>
</tr>
</tbody>
</table>

3.2 Experimental procedure

Using continuous sampling each individual horse was observed for an array of natural behaviours that are potentially lateralized (Chen et al., 2016). The horses were observed for 16 weeks from 7am-12pm and the direction (left or right) and the frequency of the behaviours for each animal was recorded using visual observation and an ethogram chart. Observations were recorded for frequent and rare motor behaviours, as well as social behaviours. “Frequently” occurring motor behaviours were observed and recorded a minimum of 80 and a maximum of 200 instances (behaviours 1-5), and “rarely” occurring motor behaviours and social behaviours were observed and recorded a minimum of 6 and a maximum of 50 instances (behaviours 6-12). Behavioural responses to the presentation of certain sensory stimuli were recorded during the afternoon. A total of 9 trials that involved the presentation of a given sensory stimulus (behaviours 13-14) was conducted with each horse.

3.3 Ethogram

3.3.1 Motor behaviours
1. **Standing**

Refers to the forelimb that was placed at least 30 centimeters in front of the other forelimb while grazing or standing (Wells and Blanche, 2008; Figure 2). 30 centimeters was chosen based on other studies and it represents an advanced stance compared to standing square. A separate occurrence was recorded after the horse had taken a few steps in order to allow it to readjust.

![Figure 2: Standing with left leg forward.](image)

2. **Flexing**

Refers to the hindlimb which was protracted during grazing or standing (Figure 3). A separate occurrence was recorded after the horse had taken a few steps in order to allow it to readjust.

![Figure 3: Flexing with left hindlimb.](image)

3. **Leading limb**

Refers to the foreleg that was used to take the first step when the horse initially started to move from a square foreleg position (Figure 4). A separate occurrence was recorded after the horse had stopped moving for at least 10 seconds.
Figure 4: A) Square position before moving and B) right leading leg position when movement begins.

4. Turning
Refers to the direction (left or right) a horse turned when making a turn of at least 90 degrees to one side. The turn had to take place in an open location that allowed for a choice of turning side, i.e. no walls or fences blocking one direction. A separate occurrence was recorded after the horse had taken a few steps in a straight line in order to allow it to readjust.

5. Fly swatting
Refers to the side of the horse that the horse was biting or moving in order to chase away insects from its body surface. This category was divided into 4 separate sub-categories 1) foreleg bite, 2) flank bite, 3) foreleg stomp, 4) hindleg stomp. Biting had to be one fluid rapid movement where the connection with the skin was completed within a second. Stomping included lifting of the leg with a rapid stomping motion without any foreword movement. Each occurrence was recorded as a separate data point.

6. Autogrooming
Refers to the side of the horse that was being groomed with the head or mouth. The grooming had to last more than 3 seconds with a direct connection to the skin. This category was subdivided into whether 1) the foreleg or 2) the flanks were groomed. Once the horse had stopped grooming for a 10 second period, then a separate grooming occurrence was recorded.
7. Defecating tail side

Refers to the side the tail was lifted when the horse defecated (Figure 5). This behaviour could be demonstrated to the left, right, or center relative to the horse’s midline. Each individual defecation was recorded as a separate occurrence.

8. Lying down

Refers to the side of the horse that touched the ground first when a horse lowered itself to the ground. This behaviour was recorded anytime the horse laid down, irrespective of the behaviours after the horse touched the ground. However, these subsequent behaviours included, 1) half lying down with its head raised (Figure 6A), 2) lying down fully horizontal with its head on the ground (Figure 6B), or 3) lying down before a rolling bout. Once the horse had stood up, then a separate occurrence was recorded.

Figure 5: Defecating tail side on the left.

Figure 6: A) Lying down on the right to a half lying position and B) lying down right to a fully horizontal position (right).
9. Rubbing

Refers to the side of the horse that was pressed up and rubbed against a substrate or another individual (Figure 7). Once the horse had stopped rubbing for a 10 second period, then a separate occurrence was recorded.

![Figure 7: left-sided rubbing on substrate.](image)

10. Pawing

Refers to the leg that the horse used when scratching the ground with the tip of its hoof. The scraping had to occur a couple of times back-to-back in order to be recorded. Once the horse had stopped pawing for a 10 second period, or taken a few steps, then a separate occurrence was recorded.

3.3.2 Social Behaviours

11. Affiliative interaction

Was subdivided into a number of behaviours, including: 1) side of approach, 2) standing close, and 3) allogrooming. Side of approach refers to the side on which the horse approached a conspecific. Standing close refers to standing in a proximity of less than approximately 60 centimeters parallel to the length of another horse, e.g. head to head or nose to head, for at least 10 seconds while grazing or resting (Figure 8A). A new occurrence of side of approach and standing close was recorded after the closest part of the horse pair actively separated by at least one-horse length. Allogrooming refers to the side of the body that was closest to the other
individual that was being groomed (Figure 8B). Once the horse had stopped grooming for a 10 second period, then a separate occurrence was recorded.

Figure 8: Affiliative interactions: A) White horses standing close as indicated on the picture and B) white horse is allogrooming on the left.

12. Agonistic interaction

Refers to the side of the body that was closest to the other horse in situations of agonistic interaction. No side was recorded in situations were the approach was head on. These situations included approaches with the ears pinned back and the neck extended, threats to bite or kick, bites, kicks or chases. Each individual instance of agonistic interaction was recorded as a new occurrence.

3.3.2 Responses to sensory stimuli

13. Auditory stimuli

A Sony 4GB Digital Voice Recorder was used to record and play sound clips of two horses neighing: 1) unknown horse from different herd and 2) known horse from the same herd. The same unknown and known sounds were used for all 9 trials of all 10 horses. The sound was played by an observer standing at a distance of 3 meters directly behind the midline of the horse. The horse was placed in cross ties in the hallway of the barn. The ties allowed for the horse to turn its ears and head to respond to the stimuli while maintaining the body in place. The observer waited for both ears to be facing forward for at least three seconds in a neutral position before playing the sound clip (Figure 9A). Each horse was subject to nine sessions during which the horses were presented with both sounds in a random order with an inter-trial of 4 minutes between sounds, and no more than one session per day. The side of the ear or head that was initially turned in relation to the sound was recorded, meaning there were 3 behavioural responses possible, 1) turning one ear backwards, 2) turning both ears backwards at the same time, and 3) turning the head to the side (Figure 9A-D). If no response was observed, then no side was recorded for that trial and the trial was repeated at the end of the study.
14. **Olfactory stimuli**

Small cube-shaped plastic containers (4cm x 4cm x 4cm) were used to present each of 3 different fecal odors: 1) unknown stallion, 2) unknown mare, 3) sheep. The container was filled with approximately 15 millilitres of freshly collected fecal sample and was presented to the midline of the horse about 2 centimeters from the horse’s nose. The horses were presented with each odor sample for a total of 9 times, with no more than 2 odors presented in a random order per day. The odors were presented every two days to avoid the horses becoming accustomed to them. The hand that was used to present the sample was rotated in order to not influence the results. The side of the nostril that was used to first inspect the odor sample was recorded. Results were recorded as either left nostril, no nostril side, or right nostril (Figure 10).

3.4 **Data analysis**

The two-tailed binomial test was used for assessing whether an animal displayed a significant side preference with a given behaviour. The chi-square test was used for assessing whether the horses as a group displayed a significant preference for one side with a given behaviour. The
same test was also used for assessing whether an animal displayed a significant side preference in behavioural responses to the presentation of sensory stimuli where the options included left, right, or no-side/center. However, data analysis was done using only the left and right data points, not the “center” data points. The chi-square test was also used for assessing whether left to right ratios significantly differ from each other.

In the literature on lateralized behavior it is common use to employ the term “correlation” when referring to an assessment of whether two lateralized behaviors, recorded in the same population of animals, are congruent with each other. Meaning that that they go towards the same side, at a rate that is above chance level. A chi-square test was used to analyze these correlation between behaviours. The chi-square tests were run both using a “strict” criterion and a “liberal” criterion. The strict criterion only considered those animals that showed a statistically significant preference for a given behaviour, whereas the liberal criterion considered all ten horses and simply defined that any observed ratio that is not exactly “fifty-fifty” indicated a “preference” for one side. Only those behaviours were tested for possible correlations which appear logical, e.g. behaviours that both included limbs, or behaviours that both belonged to the same category e.g. motor, social or sensory.

4. Results

4.1 Motor behaviours

4.1.1 Frequently occuring motor behaviours

Two horses had a significant preference for standing with the right forelimb in front and the six remaining horses had no significant side preference for this behaviour (Table 2).

Two horses significantly preferred flexing their left hindlimb while standing, while three horses had a significant right-side preference, and the five remaining horses had no significant side preference for this behaviour (Table 2).

Two horses displayed a significant preference for using their left forelimb as the leading limb when starting to move, while two horses had a significant right-side preference, and the six remaining horses had no significant side preference for this behaviour (Table 2).

Three horses had a significant preference for turning towards the left side while moving, while the seven remaining horses had no significant side preference for this behaviour (Table 2).

Two horses significantly preferred biting their left foreleg, while the eight remaining horses had no significant side preference for this behaviour (Table 2).
One horse significantly preferred **biting** its left **flank**, while one horse significantly preferred biting its right flank, and the eight remaining horses had no significant side preference for this behaviour (Table 2).

Two horses had a significant preference for **stomping** with their left **foreleg**, while the eight remaining horses had no significant side preference for this behaviour (Table 2).

One horse had a significant preference for **stomping** with their left **hindleg**, while one horse had a significant right-side preference, and the eight remaining horses had no significant side preference for this behaviour (Table 2).

Considering the eight frequently occurring behaviours together, none of the ten horses displayed a significant preference for the same side in all or at least in the majority of behaviours. No significant side preference for “frequent” motor behaviours was displayed by the horses at the population level.

**Table 2:** Individual frequencies of left- and right-side occurrence of the “frequent” motor behaviours.

<table>
<thead>
<tr>
<th>Horses/Behaviour</th>
<th>Standing</th>
<th>Flexing</th>
<th>Leading limb</th>
<th>Turning</th>
<th>Fly swatting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norton</td>
<td>32:58**</td>
<td>60:48</td>
<td>44:56</td>
<td>63:40*</td>
<td></td>
</tr>
<tr>
<td>James</td>
<td>41:45</td>
<td>45:46</td>
<td>53:47</td>
<td>52:52</td>
<td>60:74</td>
</tr>
<tr>
<td>SunDance</td>
<td>49:42</td>
<td>40:55</td>
<td>48:52</td>
<td>51:50</td>
<td>91:78</td>
</tr>
<tr>
<td>Munchy</td>
<td>37:49</td>
<td>14:66**</td>
<td>78:24**</td>
<td>58:36*</td>
<td>20:31</td>
</tr>
<tr>
<td>Sunny</td>
<td>32:71**</td>
<td>64:59</td>
<td>58:42</td>
<td>68:45*</td>
<td>78:53*</td>
</tr>
<tr>
<td>JellyBean</td>
<td>44:50</td>
<td>42:12**</td>
<td>21:81**</td>
<td>59:41</td>
<td>52:52</td>
</tr>
</tbody>
</table>

Meaning of ratio = LEFT:RIGHT

**Bold** = significant side preference.  * = p < 0.05   ** = p < 0.01

**4.1.2 Rarely occurring motor behaviours**

One horse significantly preferred **autogrooming** its left **foreleg**, while the nine remaining horses had no significant side preference for this behaviour (Table 3).

One horse displayed a significant preference for **autogrooming** its left **flank**, while

Seven horses had no significant side preferences for **autogrooming** its **flank** and three horses did not provide enough data points (Table 3).

**Defecating tail side** was analysed by comparing the number of left and right occurrences only.

Six horses had a significant preference for lifting their tail on the left side when defecating, while one horse had a significant right-side preference, the remaining three horses had no significant side preference for this behaviour, preferring the center (Table 3). Results relating
defecating tail side to sex were observed. Of the 10 horses, three males demonstrated a significant center preference. Of the significant lateral preferences, all 6 horses that preferred the left side were male. Five of these six horses occasionally lifted their tails to the center, but not to the right. The only horse that significantly preferred the right side was female (she lifted her tail to the right or center, but never to the left side; Table 3).

Seven horses displayed no significant side preference for lying down, while the three remaining horses did not provide enough data points (Table 3).

One horse significantly preferred rubbing the left side of its body, while eight horses had no significant side preference for rubbing, and the two remaining horses did not provide enough data points (Table 3).

Two horses had no significant side preference for pawing and the remaining eight horses did not provide enough data points (Table 3).

Considering the six rarely occurring behaviours together, none of the ten horses displayed a significant preference for the same side in all or at least in the majority of behaviours. No significant side preference for “rare” motor behaviours was displayed by the horses at the population level.

**Table 3**: Individual frequencies of left- and right-side occurrence of the “rare” motor behaviours and left, center, right occurrences for defecating tail side.

<table>
<thead>
<tr>
<th>Horses/Behaviours</th>
<th>Autogrooming</th>
<th>Defecating tail side</th>
<th>Lying down</th>
<th>Rubbing</th>
<th>Pawing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Foreleg</td>
<td>Flanks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norton</td>
<td>11:3</td>
<td>4:6</td>
<td>09:04:00**</td>
<td>11:8</td>
<td>2:5</td>
</tr>
<tr>
<td>Guinness</td>
<td>20:06*</td>
<td>05:05</td>
<td>16:00:00**</td>
<td>0:0</td>
<td>2:1</td>
</tr>
<tr>
<td>James</td>
<td>16:16</td>
<td>12:10</td>
<td>16:04:00**</td>
<td>7:9</td>
<td>14:18</td>
</tr>
<tr>
<td>SunDance</td>
<td>25:25</td>
<td>25:15</td>
<td>20:01:00**</td>
<td>7:6</td>
<td>6:4</td>
</tr>
<tr>
<td>Loki</td>
<td>17:21</td>
<td>11:07</td>
<td>00:28:00</td>
<td>15:08</td>
<td>5:3</td>
</tr>
<tr>
<td>Munchy</td>
<td>13:15</td>
<td>06:04</td>
<td>01:18:00</td>
<td>1:1</td>
<td>19:15</td>
</tr>
<tr>
<td>Mickey</td>
<td>5:10</td>
<td>2:1</td>
<td>32:03:00**</td>
<td>1:1</td>
<td>0:3</td>
</tr>
<tr>
<td>Sunny</td>
<td>28:30</td>
<td>1:0</td>
<td>19:15:0**</td>
<td>6:3</td>
<td>8:6</td>
</tr>
<tr>
<td>JellyBean</td>
<td>14:18</td>
<td>2:0</td>
<td>00:19:11**</td>
<td>3:9</td>
<td>4:2</td>
</tr>
<tr>
<td>Rolo</td>
<td>16:9</td>
<td>9:11</td>
<td>00:12:00</td>
<td>6:3</td>
<td>8:2</td>
</tr>
</tbody>
</table>

Meaning of ratio = LEFT:RIGHT. Meaning of defecating tail side = LEFT:CENTER:RIGHT

**Bold** = significant side preference. * = p < 0.05  ** = p < 0.01

*Italic* = not enough data points (n < 6)
4.2 Social behaviours

None of the ten horses displayed a significant side preference for approaching a conspecific or for standing close to a conspecific (Table 4).

Two horses had a significant preference for allogrooming the left side of a conspecific, while Seven horses had no significant side preference for this behaviour, and the remaining horse did not provide enough data points (Table 4).

Not enough data were collected for the agonistic behaviours for any of the horses i.e. threat, bite, kick and chase.

Considering the seven social behaviours together, none of the ten horses displayed a significant preference for the same side in all or at least in the majority of behaviours. No significant side preference for social behaviours was displayed by the horses at the population level.

Table 4: Individual frequencies of left- and right-side occurrence of the social behaviours.

<table>
<thead>
<tr>
<th>Horses/Behaviours</th>
<th>Affiliative interaction</th>
<th>Agonistic behaviours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Side of approach</td>
<td>Standing close</td>
</tr>
<tr>
<td>Norton</td>
<td>6:4</td>
<td>5:5</td>
</tr>
<tr>
<td>Guinness</td>
<td>6:2</td>
<td>7:3</td>
</tr>
<tr>
<td>James</td>
<td>5:7</td>
<td>13:10</td>
</tr>
<tr>
<td>SunDance</td>
<td>7:7</td>
<td>6:8</td>
</tr>
<tr>
<td>Loki</td>
<td>9:18</td>
<td>27:28</td>
</tr>
<tr>
<td>Munchy</td>
<td>5:8</td>
<td>8:17</td>
</tr>
<tr>
<td>Mickey</td>
<td>12:14</td>
<td>33:19</td>
</tr>
<tr>
<td>Sunny</td>
<td>8:8</td>
<td>11:12</td>
</tr>
<tr>
<td>JellyBean</td>
<td>4:6</td>
<td>1:7</td>
</tr>
<tr>
<td>Rolo</td>
<td>5:4</td>
<td>0:3</td>
</tr>
</tbody>
</table>

Meaning of ratio = LEFT:RIGHT

**Bold** = significant side preference.  * = p < 0.05   ** = p < 0.01

*Italic* = not enough data points (n < 6)

4.3 Sensory behaviours

None of the ten horses displayed a significant side preference for listening to auditory stimuli of unknown horses or for listening to auditory stimuli of known horses (Table 5).

None of the ten horses had a significant side preference for smelling olfactory stimuli of stallion feces or for smelling olfactory stimuli of mare feces (Table 5).

Two horses had a significant preference for smelling olfactory stimuli of sheep feces with the right nostril, and the remaining eight horses had no significant side preference for this behaviour (Table 5).
Considering the five sensory behaviours together, none of the ten horses displayed a significant preference for the same side in all or at least in the majority of behaviours. No significant side preference for sensory behaviours was displayed by the horses at the population level.

**Table 5:** Individual frequencies of left-, center, and right-side occurrence of the sensory behaviours. N = 9

<table>
<thead>
<tr>
<th>Horses/Behaviours</th>
<th>Auditory stimuli</th>
<th>Olfactory stimuli (feces)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unknown horses</td>
<td>Stallion</td>
</tr>
<tr>
<td></td>
<td>Known horse</td>
<td></td>
</tr>
<tr>
<td>Norton</td>
<td>4:4:1</td>
<td>1:2:6</td>
</tr>
<tr>
<td>Guinness</td>
<td>3:4:2</td>
<td>2:4:3</td>
</tr>
<tr>
<td>James</td>
<td>2:4:2</td>
<td>1:8:1</td>
</tr>
<tr>
<td>SunDance</td>
<td>4:4:1</td>
<td>0:8:1</td>
</tr>
<tr>
<td>Loki</td>
<td>0:8:1</td>
<td>1:5:3</td>
</tr>
<tr>
<td>Munchy</td>
<td>4:3:2</td>
<td>0:7:2</td>
</tr>
<tr>
<td>Mickey</td>
<td>2:1:6</td>
<td>0:7:2</td>
</tr>
<tr>
<td>Sunny</td>
<td>2:5:4</td>
<td>0:7:2</td>
</tr>
<tr>
<td>JellyBean</td>
<td>3:3:3</td>
<td>4:3:2</td>
</tr>
<tr>
<td>Rolo</td>
<td>2:7:0</td>
<td>1:6:2</td>
</tr>
</tbody>
</table>

Meaning of ratio = LEFT:RIGHT:CENTER
**Bold** = significant side preference. * = p < 0.05  ** = p < 0.01

**4.4 Consistency of side preferences across behaviours**

No consistency of side preferences across behaviours was found for any of the ten horses. All horses demonstrated a task-dependent behavioural lateralization.

**4.5 Correlations between behaviours**

See Section 3.4 Data analysis for definition of “correlation” in the present study.

**4.5.1 Correlation analysis for standing, flexing, leading limb and turning**

No significance was found for correlation analyses run between: 1) standing and leading limb, 2) leading limb and flexing, 3) leading limb and turning, and 4) standing and flexing.

**4.5.2 Correlation analysis for autogrooming and fly swatting**

No significance was found for correlation analyses run between: 1) autogrooming foreleg and foreleg bite, 2) autogroom foreleg and foreleg stomp, 3) autogroom flank and flank bite, 4) foreleg bite and flank bite, and 5) foreleg stomp and hindleg stomp.

**4.5.3 Correlation analysis for affiliative behaviours**

No significance was found for correlation analyses run between: 1) approach and stand close, 2) approach and allogrooming, and 3) stand close and allogrooming.
4.5.4 Correlation analysis for sensory behaviours

Not enough data were collected to run correlation at the strict level. No significance was found for correlation analyses run at the liberal level between, 1) auditory known and auditory unknown, 2) olfactory male and olfactory female, 3) olfactory male and olfactory sheep, and 4) olfactory female and olfactory sheep.

4.5.5 Other behaviours

Not enough data were collected to run correlations on agonistic behaviours. No correlation analyses were run on defecating tail side, lying down, rubbing and pawing.

5. Discussion

The results of this study demonstrate that horses display 1) significant side preferences for some behaviours at the individual level, which can be observed as task-dependent changes in their side preferences, 2) no significant side preferences at the population level, and 3) no significant correlations between the studied behaviours.

5.1 Own findings

The current study was the first to assess possible side biases in a large variety of behaviours in a given population of horses. Although no significant side preferences at the population level were found with any of the seventeen behaviours considered, most of the horses displayed significant side preferences at the individual level for a variety of behaviours. There are certain noteworthy observations that will be analysed here: defecating tail side and lying down behaviours.

Defecating tail side is not a commonly studied behaviour in horses. There are three options for tail movement when defecating: left, center and right. In this study, males never preferred the right side, rather preferring the left or center. The female never defecated with her tail to the left side, rather she defecated to the center or right side, with a right-side lateralization. Further studies could help identify whether the lateralization of defecating tail side is statistically indicative of a population level side preference or affected by sex.

This study was also one of the first to collect enough data on possible side preferences for lying down. No significant side preference was found for this behaviour. I hypothesise that the unlevel ground could have an impact on this behaviour since the study location was not completely flat with rolling hills. The horses may have lied down on whichever side was more comfortable due to the level of the ground. However, horses could have just rotated direction to lay on their preferred side. Therefore, other factors such as herd direction, sun, or shade could
have impacts on this lateralization. Future studies should observe this behaviour on a level surface.

5.2 Comparison with previous studies in horses

Studies have reported that individual and population level side preferences in horses can be impacted by breed, age, sex, training, handling, and arousal (Cully et al., 2018). These factors were not taken into account during the present study due to sample size. The only study so far which assessed possible correlations between motor and sensory behaviours failed to find any (Farmer et al., 2018).

5.2.1 Standing

McGreevy and Rogers (2005) found that thoroughbred racehorses demonstrate a population bias to place the left forelimb in front of the other when grazing. In comparison, Austin and Rogers (2012) found no standing preference in feral horses, which may indicate that the observed preference may be a consequence of training or may be due to breed. In the English riding style, horses are always worked with from the left side. For example, horses are approached, led, and mounted on the left side. Therefore, they may be accustomed to using the left side and therefore trained horses may have a different lateralization compared to feral, untrained, horses. The current study found that 20% of the horses had a significant preference for standing with the right foreleg in front and 80% had no preference for an extended leg when standing. The difference in the preference side between this study and the study by McGreevy and Rogers (2005) could be in relation to the breed of the horse. The present study had a variety of breeds, whereas, the study by McGreevy and Rogers (2005) only looked at thoroughbred racehorses. Standing side preference could be influenced by breed or training purposes. Future studies should analyze the impact of breed on standing lateralization.

5.2.2 Flexing

McGreevy and Rogers (2005) observed the laterality of flexing in horses: 12% showed a preference for flexing the left hindleg, 8% showed a preference for flexing the right hindlimb, and, the remaining 80% showed no preference for flexing the hindlimb. In contrast, the present study found that 50% of the horses had a significant preference for hindlimb flexing (30% right and 20% left) and 50% did not demonstrate a significant preference for either hindlimb. This discrepancy between studies may be reflective of the different horses, for example sex, age and breed, in each group and their individual behavioural lateralities.
5.2.3 Leading limb
Cully et al. (2018) studied the leading limb of 2,095 thoroughbred horses and determined that 51.3% led with their left leg, and 48.7% led with their right leg when stepping off from a square position. Similarly, Grzimek (1968) found that 52.5% of thoroughbred horses began to walk with their right foot, 40% with their left and 7.5% having no preference, whereas Williams and Norris (2007) determined a population level laterality among 9,116 racehorses to initiate running with the right foot (90.2%). The results of the current study are in line with the finding of Cully et al. (2018) and Grzimek (1968) where initial foot preference was split between left (20%) and right (20%) preference.

5.2.4 Defecating tail side
This behaviour has not been studied so far in horses as researchers have focused on more obvious behaviours such as motor, sensory and more recently social interaction. This behaviour was included during the present study since it can clearly be observed with the human eye as a right, center, or left side preference.

5.2.5 Autogrooming, rolling, rubbing, lying down, and pawing
Most studies of horse laterality so far focused on the more frequently occurring motor patterns and responses to sensory stimuli. Very little research has been done on the “rare” behaviours that are possibly lateralized in horses and therefore included in the present study. McGreevy and Rogers (2005) collected data over a 2-hour period to assess possible side preferences in certain rare behaviours such as grooming forelegs with head or mouth, grooming flanks with mouth, rolling, rubbing on substrates, and lying down. However, pawing was the only behaviour for which enough data were collected. The authors did not find any significant side preferences but determined that pawing was significantly affected by age, but not by sex, being more frequent in two-year-olds than in any of the other age groups. This may indicate that pawing is a behaviour more commonly seen in younger horses which may explain why it was rarely observed during the present study in which the youngest horse was 12 years of age.

5.2.6 Affiliative and agonistic interactions
Farmer et al. (2018) focused on the occurrence of side preferences in the affiliative interaction and studied thirty-one animals in four groups of horses and ponies of different breeds and social composition. A significant preference was found for the left side in affiliative approaches and interaction. In contrast, the present study did not find a statistically significant side preference in the affiliative behaviours of approach side at the population level or standing close.
Research on lateralized behaviour in social interactions has so far focused on agonistic behaviours, stressful situations and negative emotions in which a consistent left-side preference or right brain hemisphere utilization was found (Austin and Rogers, 2012; Farmer et al., 2018). Austin and Rogers (2012) found a left-side population bias in feral horses during agonistic interactions. In contrast, the present study did not collect enough data to analyze agonistic lateralization. This may be the result of the large pasture size. If the horses did not like each other then they had enough room to keep away from each other.

5.2.7 Auditory stimuli
When a sound is played back a horse can orient its head and ears either independently or simultaneously towards the sound source (Basile et al., 2009). Although the vocal repertoire of horses is limited, they are able to discriminate between whinnies of different individuals (Basile et al., 2009). This ability allows the test of auditory laterality between whinnies of known and unknown individuals. Basile et al. (2009) tested the influence of the degree of familiarity on side preferences in the auditory response in domestic horses and found a right ear preference for processing calls of familiar conspecifics and no bias for processing the call of strangers. The present study is consistent with the findings of Basile et al. (2009) with regards to the call from strangers where no horses had a preference for a call from an unknown horse. However, the present study also found no side preference for a call from a known individual. The relatively low number of data points collected per animal might explain the lack of significant side preferences in the present study.

5.2.8 Olfactory stimuli
Horses have a highly developed sense of smell and are able to identify other horses by their smell (McGreevy and Rogers, 2005). McGreevy and Rogers (2005) observed nostril preferences of 157 horses by presenting them with stallion feces in a plastic bag and found a significant preference for horses under the age of four years to use their right nostril to smell stallion feces, however, no nostril preference was found in older horses. The authors presented a sub-set of 78 horses with the olfactory stimuli on two consecutive days to determine the consistency in responses to the same olfactory stimulus. The correlation between nostril use on the first and second day was mild but significant (Pearson correlation $r = 0.27$, $P = 0.05$) (McGreevy and Rogers, 2005). The authors concluded that the olfactory test is more reliable when novelty is high (McGreevy and Rogers, 2005). The current study used a somewhat different approach, by presenting the feces in a container for a total of 9 times with at least a day between exposures. This extra day between trials compared to McGreevy and Rogers
was in order to try and reduce the possibility of habituation and thus the possibility of the number of center results. The scent was first approached to the midline of the horse, then the horses had the possibility to continue smelling the sample or simply stop smelling and turn away. When horses wanted to smell deeper and longer, they would choose a side, either right or left. However, when a center trial was recorded it often meant the horse smelt the fecal sample quickly and then turned away without investigating further. This could demonstrate that the horses did not want to investigate the scent more thoroughly and get closer with one nostril. This could be attributed with age, where the younger horses are more curious and thus investigate further compared to older horses. Further studies should test the correlation between age and presence of olfactory lateralization in more detail.

5.2.9 Correlations between behaviours

Studies on lateralized behaviours in horses have so far only assessed one or two behaviours at a time. It is currently not known whether certain lateralized behaviours observed in horses may correlate with each other due the lack of studies that have assessed a wide range of potentially lateralized behaviours in a given study population. McGreevy and Rogers (2005) assessed a possible correlation between the foot preference when standing or grazing with the first nostril used to smell, however, no significant correlation was found between those behaviours. Similarly, no significant correlation was found between those two behaviours in the present study. However, there is little reason to believe that a motor pattern performed by the front limb and a sensory response performed by part of the snout should be correlated with each other. The present study performed correlational analyses between behaviours that were more likely to correlate with each other, e.g. behaviours which both included limb use. However, the study failed to find any significant correlations. More research on lateralized horse behaviour correlation should be done in order to draw more conclusions. With that in mind, however, correlations should focus on behaviours or sensory responses that are likely to have a common neuroanatomical substrate or a common functional background.

5.3 Comparison with previous studies in other mammals

The level of behavioural lateralization of species can fall anywhere on a broad spectrum ranging from significant side preferences at the population level which are consistent across behaviours at one end, to a complete lack of side preferences even at the individual level at the other end. Lateralization of brain function has been extensively studied in mammals, particularly humans. The human brain tends to have left-lateralized functions such as language and motor abilities
and right-lateralized functions such as visuospatial attention (Gotts et al., 2013). Human handedness is the most commonly known behavioural lateralization at the population level where 90% of people display a consistent right-hand preference across a wide variety of manual motor patterns and tasks.

Due to the close phylogenetic relationship with human, studies on lateralized behaviours in the animal kingdom have so far mainly focused on nonhuman primates. Certain primate species show a population-level bias for highly complex tasks, where the complexity of a given task has been recognized to influence the magnitude, consistency and direction of hand preference in great apes (Meguerditchian et al., 2010). Therefore, the use of a preferred hand at the population level may only be seen in highly complex tasks involving bimanual action (Tabiowo & Forrester, 2013). Byrne and Byrne (1991) determined that mountain gorillas have a significant right-hand bias in bimanual actions involving feeding.

Compared to the studies of lateralized behaviour in nonhuman primates, the studies on species such as ungulates are scarce. A review article on all aspects of laterality research on ungulate livestock by Leliveld (2019) included a total of 132 articles that fit the requirement set by the review. The majority of the articles focused on horses and cattle (57 and 37 articles, respectively); fewer studies focused on sheep, goats, pigs and donkeys (20, 8, 7, and 4 articles), one study was completed on buffalo, and no studies were completed on camelids or mules (Leliveld, 2019). Significant side biases in the behaviour of non-human vertebrates at the population level are extremely rare, and significant findings at the individual level are dependent on the study and are usually task-dependent (Leliveld, 2019). Studies on behavioural lateralization of sound perception of conspecific vocalization in non-human vertebrates by Ocklenburg et al. (2013) propose a left hemispheric dominance. However, the few studies conducted to date on horses and goats do not support this generalization (Leliveld, 2019).

Horses and ungulates in general do not seem to be behaviourally lateralized at the population level compared to humans and some great apes, however, they show some data-supported task dependent individual lateralization. Additional studies need to be conducted in order to establish more clear information on horse behavioural lateralization since many papers are contradictory in the presence of individual lateralization in tasks.

5.4 Theoretical context

Hemispheric specialization occurs when certain functions of the brain are predominantly or even exclusively present on one side of the brain. This specialization can manifest itself as lateralized behaviour.
At the individual level, having a lateralized brain is an efficient way to increase neural capacity and efficiency without increasing brain mass and also allows for the ability to multitask (Rogers and Vallortigara, 2015; Hopkins and Cantalupo, 2008). However, it can also be disadvantageous for individuals to be lateralized when a stimulus is equally likely to appear on either side of the body (Dharmaretnam and Rogers, 2005). For example, if the field of vision is lateralized it can take longer to detect a predator on the non-preferred side (Lippolis et al., 2002). This example of visual lateralization being potentially disadvantageous is present in horses, where the eyes are positioned laterally with only 60°-70° binocular vision in the front, up to 205° monocular vision on the sides and a blind spot behind the horse (De Boyer Des Roches et al., 2008). Lateralization of vision would create a disadvantage for predator detection in the monocular field.

At the population level, behavioural lateralization can be advantageous since it allows group-living animals to stay together. For example, turning preference allows shoal living fish to keep together when avoiding a predator (Rogers and Vallortigara, 2005). On the other hand, having a population lateralization for behaviours such as predator-avoidance can cause individuals, specifically prey species, to become predictable to predators (Vallortigara, 2006). Horses are social animals. The herd is usually led by an older mare, referred to as the alpha mare, and the rest of the herd is composed of one or two stallions, females and young (Linklater, 2000; Warning, 2002). In the event of danger, the alpha mare will lead the herd away. Compared to shoal living fish that are lateralized at the population level to stay together, horses are not lateralized at the population level for the direction of escape turning (Austin and Rogers, 2007). Horses do not need to rely on lateralization to stay together as they follow the alpha mare and they avoid becoming predictable to predators as a consequence of not exhibiting population lateralization. Social structure, i.e. the presence of an alpha mare, may be a reason that population lateralization did not evolve in motor behaviours such as turn side, a behaviour relevant to predator avoidance.

Brain lateralization has both advantages and disadvantages and has evolved differently depending on the needs of the species. When considering horses, the lateralized body morphology, complex social hierarchy, and group composition may explain the lack of population level lateralization. The lack of population level lateralization in the present study would support the idea that individual lateralization is more advantageous then population lateralization for horses. In other words, lateralization would not be as advantageous to horses as it is for other species. Future research should collect more expansive data on sensory literalities on a wider range of horses and evaluate for other behavioural correlations.
6. Societal & ethical considerations

The study reported here complies with the European Union Directive On Animals Used For Scientific Purposes (EU directive 2010/63/EU) and with the requirements of the Animals For Research Act of Ontario, as amended 1976, and the recommendations of the Canadian Council for Animal Care (CCAC). As the study only involved observations of spontaneous behaviour and observations of behavioural responses to the presentation of natural stimuli, no extra ethical approval was required.

The horse owner, Barb Malcom, offered her horses for participation in the non-invasive observation and sensory testing. She was informed and agreed with the test procedures and the intended publication of the data prior to the beginning of data collection. The data collection did not cause the horses any pain, suffering, or damage.

The increased knowledge of behavioural laterality in domesticated animals, such as horses, may contribute to stress reduction during human-animal interactions, improved health assessments, production, and welfare of such animals in captivity.

If it was determined that horses have a side preference or lateralization for certain behaviours, this could allow owners to interact with their domestic horses in ways that reduce stress during human-animal interactions, for example when approaching a horse. This could also help during training of young horses. Performing tasks and moving around the young horse in a way that makes it most comfortable for them could reduce the stress of training and of novel interactions. In addition, when working horses are often used in pairs to pull farm equipment or trailers. If it is known that certain horses prefer to be on a specific side of another horse, then placing pairs together on specific sides could increase production and lower stress to the animals during work and training. With this in mind, learning how to determine the side preference or lateralization of horses could allow training techniques to be adapted. For example, training in the English riding style is done from the left side, but perhaps this is not the best and most ethical way of training all horses and it may need to be updated. Knowing the laterality of certain horse behaviours could also improve health assessments. If horses display a certain lateralization or lack of lateralization for a certain behaviour, then a sudden change in this behaviour could be an indication of disease or pain. For example, if a certain horse has a preference for leading limb and standing on a certain leg, then a sudden change in this behaviour could indicate a tenderness or injury that needs to be addressed.

All of the examples listed above are ways in which knowledge of lateralized behaviours of horses could increase the welfare of such animals in captivity. Learning more about the natural behaviour of species can only serve to increase their welfare while in our care.
7. Acknowledgements

I would like to thank Barb Malcom, owner of Churchill Chimes Equestrian Center, as well as other barn staff, mainly Robin Purchase for giving me permission to conduct the study and for field assistance. I would like to acknowledge the field assistance of Vivianne Stewart, Alexander Gabbour, and Andrew Rick, and the continued support of my parents and supervisor, Matthias Laska.

8. References


