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The Influence of Pointing Accuracy in an Object-Choice Task with Domestic Horses *(Equus caballus)*

by

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of the requirements for the degree of
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Abstract

Domestic horses (*Equus caballus*) are known to successfully engage in human social cue-following tasks, and exhibit signs of behavioral flexibility based on human attentional state and body language and individual memory retention. In this study, eight domestic horses from the Ramapo Equestrian Center in Suffern, New York were used in an object-choice task in order to evaluate the influence of pointing accuracy and experimenter identity on subsequent horse behavior. All horses completed two experimental phases and were evaluated based on their ability to follow experimenter cues (defined as "obedience") and latency to approach buckets within individual trials. In phase one, one experimenter was responsible for conducting a set of six trials in which they provided accurate pointing cues towards a baited bucket twice (set A), provided inaccurate pointing cues towards an empty bucket twice (set B), and provided accurate pointing cues towards a baited bucket twice (set C). After a one-week hiatus, the same experimenter conducted phase two, in which they administered sets D and E, which were identical to sets A and B, but then the experimenter was replaced by a second experimenter who accurately baited a bucket twice (set F). Horses followed cues reliably in sets A and D, and showed a trend for reduced obedience between sets A and C, yet not between sets D and F. This indicates that horse responses were likely due to the source of inaccurate cueing, and more specifically, the familiarity of the experimenter (which likely shaped how the horses perceived the inaccurate cues in set B). Responses also point to evidence of learning and memory retention, since horses only followed pointing cues during their first set of inaccurate pointing cues. Results are discussed in reference to human-animal relationships, memory retention and behavioral flexibility.

Keywords: Social cue-following, behavioral flexibility, domestic horses, object-choice task.

The Influence of Pointing Accuracy in an Object-Choice Task with Domestic Horses (*Equus caballus*)

The ability for animals to communicate varies across species and is heavily influenced by societal and genetic constraints (Melis, Hare, & Tomasello, 2006). In its most basic form, conspecific communication enables humans and select animal species to convey and process information, such as sources for food and immediate threats (Tomasello, Call, & Hare, 1998). More sophisticated forms of communication, including cross-species cue following and problem-solving (Hare, Brown, Williamson, & Tomasello, 2002; Hare & Tomasello, 2005), tend to arise in domesticated species due to familiarity with human signals and selection for traits that facilitate the human-animal relationship (Hare et al., 2002; Kaminski, Riedel, Call, & Tomasello, 2005; Proops, Walton, & McComb, 2010). Nevertheless, even across these species, large variability exists (McKinley & Sambrook, 2000) and has sparked further investigations into species' relative stages of domestication (McKinley & Sambrook, 2000; Kaminski et al., 2005; Proops et al., 2010), signal integration (Kundey, De Los Reyes, & Arbuthnot, 2010; Smith, Wilson, McComb, & Proops, 2018; Takaoka, Maeda, Hori, & Fujita, 2015) and the scope of the human-animal relationship (Maros, Gacsi, & Miklosi, 2008; McKinley & Sambrook, 2000).

Domestic horses (*Equus caballus*) represent a unique model for investigating the impacts of domestication and human interactions on cue-following due to their comparatively late domestication (Ludwig, et al., 2009) and low frequency of human contact relative to household pets (McKinley & Sambrook, 2000; Ringhofer & Yamamoto, 2017). Household pets, such as domestic dogs (*Canis familiaris*), have received greater attention in human-mediated tasks, and are consistently successful in deciphering human gestures within cognitive experiments (Cook, Arter & Jacobs, 2014; Hare & Tomasello, 2005; Kundey et al., 2010; McKinley & Sambrook,

2000; Takaoka et al., 2015). Further elucidating social cue-following in domestic horses may help identify the point in the domestication process at which this trait emerged, and how human relationships shaped this development.

Investigations into these domestication processes allow researchers to create a timeline for the onset and utility of modern traits and further decipher the complex human-animal relationship. While the earliest recorded domestication involving dogs occurred between 15,000 and 40,000 years ago (Thalmann et al., 2013; Savolainen, Zhang, Luo, Lundeberg, & Leitner, 2002), it was not until approximately 5,500 years ago that the domestication of modern horses began (Ludwig, et al., 2009). Nevertheless, across all species, domestication, achieved by a combination of genetic and environmental factors (Price, 1984), tends to prioritize "the reproduction of a deme (i.e. local sub-population) of animals or plants . . . for material, social or symbolic profit" (Vigne, 2011, p. 172). This has been shown in wolves that were especially valued for their role in hunting, companionship and protection and were likely used as a food source (Moutou & Pastoret, 2010), and horses that assisted substantially during warfare, transportation and communication (Ludwig et al., 2009).

The convergence of genetic and environmental factors continues to influence modern-day behavioral traits in animals (Axel-Nilsson, Peetz-Nielsen, Visser, Nyman, & Blokhuis, 2015; Hare & Tomasello, 2005; Hausberger, Bruderer, Le Scolan, & Pierre, 2004) and competence within human-mediated tasks (involving cue-following, cooperation and communication), across domesticated species (Hare & Tomasello, 2005; Maros et al., 2008; McKinley & Sambrook, 2000; Miklosi, Polgardi, Topal, & Csanyi, 2000; Proops et al., 2010; Ringhofer & Yamamoto, 2017). The relative influences of domestication and learning have been the subject of much debate (Balint et al., 2015; Hare et al., 2002; Hare & Tomasello, 2005; Maros et al., 2008;

Miklosi & Soproni, 2006; Riedel, Schumann, Kaminski, Call & Tomasello, 2008; Proops, Rayner, Taylor, & McComb, 2013; Udell, Dorey, & Wynne, 2008), where some argue that domestication facilitated an environment for human-animal cooperation (Hare & Tomasello, 2005), while others posit that the social and developmental environment precipitated these adaptations (Udell et al., 2008). Ultimately, it is believed that some combination of factors, including genetic selection, environmental exposure and ontogenetic development likely contribute to domesticated animal behavior (Hausberger et al., 2004; Hausberger, Roche, Henry, & Visser, 2008; Kaminski et al., 2005; Proops et al., 2010; Ringhofer & Yamamoto, 2017).

Domesticated animal behavior is especially noteworthy in regards to social cue-following. Social cue-following has been tested through experimental, cognitive paradigms, such as the object-choice task. The object-choice paradigm often tests boundaries of interspecific communication by requiring a non-human subject to rely on human cues (visual, olfactory, auditory or vocal) in order to find food hidden in one of two locations. During these tasks, human cues often vary in salience, and can range from minute cues such as those employing momentary pointing or gaze follow (including glancing alternation, head orientation or body orientation) to more profound cues such as those involving sustained pointing or marker placement (Call, Hare, & Tomasello, 1998; Hare, Call, & Tomasello, 1998; Hare & Tomasello, 1999; Itakura & Tanaka, 1998; Kaminski et al., 2005; Maros et al., 2008; McKinley & Sambrook, 2000; Miklosi, Polgardi, Topal, & Csanyi, 1998; Nawroth, Ebersbach, & von Borell., 2014; Proops et al., 2010). These tasks are critical in providing insight into the influence of domestication (Hare & Tomasello, 2005) and environmental or social factors (Udell et al., 2008) on interspecific cue-following, as well as the sensory modalities used by various species in the wild (Call et al., 1998; Plotnik et al., 2013). Although these tests have been conducted on an

array of domesticated and non-domesticated species that vary in intelligence (see Table 1 for a

select list of object-choice task successes and failures), domesticated species tend to be highly

successful in following human gestural cues in an object-choice task (Kaminski et al., 2005,

Miklosi et al., 1998; Nawroth et al., 2014; Proops et al., 2010; Takaoka et al., 2015).

Tests of human gestural cues differ from other metrics of intelligence (Brauer, Call &

Tomasello, 2005), since they not only require an animal to decipher human cues (with which

they may lack familiarity), but also cooperate in a task to collect a food reward, which may

contradict the social norms of more competitive environments (Hermann, Melis, & Tomasello,

2006; Melis et al., 2006). Tests of interspecific cue-following with intelligent, non-domesticated

animals have thus resulted in varied success across species, including apes (*Pan troglodytes,*

Pongo pygmaeus, Pan paniscus and *Gorilla gorilla*) (Call, 2004; Brauer et al., 2005; Call et al.,

1998; Hermann et al., 2006; Itakura & Tanaka, 1998), bottlenose dolphins (*Tursiops truncatus)*

(Pack & Herman, 2004; Tschudin, Call, Dunbar, Harris, & van der Elst, 2001), Asian elephants

(*Elephas maximus*) (Plotnik et al., 2013; Plotnik, Shaw, Brubaker, Tiller, & Clayton, 2014) and

African elephants (*Loxodonta africana)* (Smet & Byrne, 2013). Specifically, bottlenose dolphins

tend to be highly proficient in following human visual cues within object-choice tasks (Pack &

Herman, 2004; Tschudin et al., 2001), while visual, object-choice tasks with elephants (likely

due to their poor visual acuity), have resulted in mixed success (Plotnik et al., 2013; Smet &

Byrne, 2013). Interestingly, despite mixed results regarding primates' proficiency in object-

choice tasks (Anderson, Sallaberry, & Barbier, 1995; Call et al., 1998; Hermann et al., 2006;

Itakura & Tanaka, 1998), in other contexts, they are known to effectively engage in visual

perspective-taking and gaze-follow with humans and conspecifics (Brauer et al., 2005;

Tomasello et al., 1998).

Table 1. *Object-choice tasks successes and failures across select species*

Species	Object-Choice Tasks						Source
	Visual		Olfactory		Auditory		
	Success	Failure	Success	Failure	Success	Failure	
Domestic dog (*Canis familiaris)*	X*^						Hare et al., 1998; Hare & Tomasello, 1999; Miklosi et al., 1998
Domestic horse (*Equus caballus)*	X^	X*				X	Maros et al., 2008; Proops et al., 2013; Proops et al., 2010
Domestic goat (*Capra hircus*)	X^	X*					Kaminski et al., 2005
Domestic pig (*Sus scrofa domestica)*	X^*						Nawroth et al., 2014
Asian elephant (*Elephas maximus*)		X^	X			X	Plotnik et al., 2013; Plotnik et al., 2014
African elephant (*Loxodonta africana)*	X^	X*					Smet & Byrne, 2013
Chimpanzee (*Pan troglodytes)*	X*^				X		Call, 2004; Call et al., 1998 (only when food is visible); Itakura & Tanaka, 1998
Orangutan (*Pongo pygmaeus)*	X*^				X		Call, 2004; Itakura & Tanaka, 1998
Gorilla (*Gorilla gorilla*)					X		Call, 2004
Bonobo (*Pan paniscus*)					X		Call, 2004
Capuchin monkey (*Cebus paella*)	X^	X*					Anderson et al., 1995
Bottlenose dolphin (*Tursiops truncatus)*	X*^						Pack & Herman, 2004; Tschudin et al., 2001

Notable examples of species' success or failure in human-mediated object-choice tasks, including visual, olfactory and auditory. X* = gaze (with or without head orientation). X^ = point (with or without gaze).

Domestic dogs display advanced acuity within object-choice tasks, as demonstrated through their abilities to follow human communicative gestures that differ in salience (Hare et al., 1998; Hare & Tomasello, 1999; McKinley & Sambrook, 2000; Miklosi et al., 1998). In a study by Miklosi et al. (1998), human experimenters provided five types of cues— pointing, bowing, nodding, head-turning and glancing – towards one of two containers in an object-choice task, and revealed that most dogs could attend to pointing, bowing and nodding cues, yet often needed additional training to attend to head-turning and glancing cues. Differences in cue salience are likely influenced by familiarity with signals and levels of training (McKinley & Sambrook, 2000; Miklosi et al., 1998). Although Hare et al. (1998) used a smaller sample size, they found that domestic dogs were unable to follow eye movements when they conflicted with head movements, yet seemed to understand the role of a human in helping them to find the food and the importance of approaching the front of a human during a communicative task, such as dropping a toy ball for the experimenter to retrieve.

Domestic horses respond to a variety of pointing signals, however, lack acuity in deciphering more nuanced signals in an object-choice task (Maros et al., 2008; McKinley & Sambrook, 2000; Proops & McComb, 2010; Proops et al., 2013; Proops et al., 2010). Pfungst (1911) first revealed the influence of subtle and unintentional human gestures on horse behavior, while more recent research has revealed communicative limitations in the human-horse relationship (Maros et al., 2008; McKinley & Sambrook, 2000; Proops et al., 2013; Proops et al., 2010). A study by Maros et al. (2008) revealed that socialized horses could respond to all pointing cues except for distal momentary pointing cues, where the gesture is discontinued prior to the horses' choice. Proops et al. (2013) argue that rather than human socialization influencing social cue following, it is more likely that horses learn to follow basic stimulus enhancement

cues (such as the human arm) in an object-choice task. In an object-choice task experiment

conducted by Proops et al. (2010), horses presented with a variety of signals, including distal

sustained pointing, momentary tapping, marker placement, body orientation and gaze alternation,

were only successful during sessions involving stimulus or local enhancement cues. Domestic

horses' use of stimulus enhancement along with their reduced success compared to dogs,

suggests a lesser degree of comprehension in tasks requiring human-animal communication

(McKinley & Sambrook, 2000; Proops et al., 2010), and the likelihood of interspecific

communicative capabilities that more closely resemble that of goats (Proops et al., 2010). Similar

to domestic horses, domestic goats rely on cues of stimulus enhancement (touching or pointing)

in an object-choice task, and are unable to rely on human gaze cues (Kaminski et al. 2005).

Interestingly, in the case of conspecific social cueing, dogs have demonstrated variable

success when required to use conspecific cues to locate a hidden food item (Balint et al., 2015;

Hare & Tomasello, 1999), while horses successfully rely on conspecifics to locate a hidden food

item (Wathan & McComb, 2014). In a study that required domestic dogs to rely on conspecific

gaze cues to locate food, less than half of the subjects performed above chance (Hare &

Tomasello, 1999), which may explain findings from Balint et al. (2015), where they argue that

due to infrequent interactions between domestic dogs, they were unsuccessful in following

momentary or sustained gaze cues provided by a video-projected conspecific. On the other hand,

since horses communicate using multiple sensory modalities including visual cues— such as eye,

ear and mouth movements (Waring, 2002), it is not surprising that they are successful in using

conspecific head orientation and ear and eye cues to locate food in an object-choice task (Wathan

& McComb, 2014).

Interspecific social cue-following tends to be highly developed in domesticated species,

which may explain patterns of communicative flexibility within problem-solving tasks (Cook et al., 2014; Kundey et al., 2010; Petter, Musolino, Roberts & Cole, 2009; Ringhofer & Yamamoto, 2017; Savalli, Resende, & Gaunet, 2016; Smith et al., 2018; Takaoka et al., 2015). This flexibility manifests in visual perspective-taking and attention to human knowledge states (Brauer, Call, & Tomasello, 2004; Call, Brauer, Kaminski, & Tomasello, 2003; Hare et al., 1998; Kaminski, Brauer, Call, & Tomasello, 2008; Kaminski, Hynds, Morris, & Waller, 2017; Ringhofer & Yamamoto, 2017) and avoidance behavior during deception (Cook et al., 2014; Kundey et al., 2010; Petter et al., 2009; Takaoka et al., 2015).

The adaptability of domestic dog behavior is dependent on training and cue-type, where they can only learn to temporarily avoid human static pointing cues following training and visible container baiting (Kundey et al., 2010) and exhibit difficulty inhibiting responses to a deceptive but familiar experimenter, even when a stranger is non-deceptive in an object-choice task (Cook et al., 2014). However, in the presence of other types of pointing cues, dogs likely utilize social adaptability traits (similar to humans) due to their shared social environment (Takaoka et al., 2015). For instance, dogs have been observed preferentially avoiding misleading cues in an object-choice task (Petter et al., 2009; Takaoka et al., 2015), and in addition to approaching a cooperative experimenter (who provides pointing cues towards a baited container) significantly more often than a non-cooperative experimenter, they also can generalize these behaviors to inanimate objects that represent the experimenters (Petter et al., 2009). Takaoka et al. (2015) further evaluated the role of experimenter identity in tasks where dogs first inhibited responses towards dishonest experimenters who pointed to an unbaited container, yet resumed above-chance response levels in the presence of a new, honest experimenter, who pointed to a baited container.

Domestic horse behavioral flexibility has been revealed through attention to human knowledge states (Proops & McComb, 2010; Ringhofer & Yamamoto, 2017), discrimination between human emotive expressions (Smith, Proops, Grounds, Wathan, & McComb, 2016) and preferential approach behavior in problem-solving tasks (Krueger, Flauger, Farmer & Maros, 2011; Smith et al., 2018). In a problem-solving task where buckets were baited in the presence of a horse, they exhibited increased visual and tactile signaling towards humans that had not witnessed the baiting (Ringhofer & Yamamoto, 2017), which has been used as support for the suggestion that horses are aware of human attentional states, understand humans' past experiences, and are able to adapt to cueing accordingly. Horses show similar flexibility when attending to signals from experimenters that involve postural differences—such as dominant or submissive (Smith et al., 2018) – and attentional differences, such as when an experimenter is focusing on the subject or elsewhere (Proops & McComb, 2010). These behavioral differences that arise from variability in human attention and gestures contribute substantially to the growing evidence of horses' plasticity within human-mediated tasks and serve to further our understanding of evolutionary influences on horse behavior (Proops & McComb, 2010; Ringhofer & Yamamoto, 2017; Smith et al., 2016; Smith et al., 2018).

Evidence of domestic horses' plasticity within interspecific, cognitive paradigms has exciting implications for understanding the human-horse relationship. By evaluating the ways in which horses interpret accurate vs. inaccurate human gestures (which have not yet been tested in the confines of an object-choice task), we can refine our knowledge of interspecific communication and reveal the scope of horses' capacity for deciphering human cues. The current study evaluates the relationship between responses to cues and experimenter accuracy in an object-choice task with domestic horses. In order to test the influence of positive or negative

experiences with an experimenter on horse behavior, two experimenters provided sustained, ipsilateral pointing cues in an object-choice task. For each horse, one experimenter was always accurate, and consistently pointed towards the baited bucket, while the other experimenter was inaccurate, and alternated pointing cues towards either the unbaited or baited bucket. This experiment intended to reveal the role of experimenter 'accuracy' and identity on subsequent horse behavior. We hypothesized that after a negative experience with an experimenter— characterized as an inaccurate pointing cue—horses would show reduced response rates and increased latencies to approach the buckets, and when presented with an accurate experimenter, horses would resume cue-following at above-chance levels and display significantly shorter latencies to approach the buckets. This study intends to inform our understanding of how horses follow social cues provided by humans, and whether the honesty of the humans' cues affect the horses' behavior. In addition, this work has implications for improving husbandry, training and management practices for captive horses.

Methods

Subjects

Thirteen domestic horses (*Equus caballus*) from the Ramapo Equestrian Center in Suffern, NY participated in this study. Eight of these horses (6 gelded males and 2 mares) completed the training criterion (Table 3) and were included in the final analysis. Horses ranged from 10 to 24 years of age (mean = 16.25, SD = 5.28). All horses were housed individually and received ad libitum access to water alongside their daily portions of grain and hay. Horses were socialized to interact with two experimenters and one handler, although the roles of these individuals were subject to change based on experimental protocol. Participants were selected from a pool of available horses at the center and were included in the final analysis pending

successful completion of training procedures. While some horses were familiar with pointing cues, none had prior exposure to the experimental protocol. All horses were either school horses (equestrian center- or privately-owned) or non-school horses. This study protocol was approved by the Ramapo Equestrian Center and the Hunter College IACUC.

Apparatus and Materials

Two, identical opaque buckets with gamma seal lids were used to conceal food rewards and avoid olfactory or visual cues. Each bucket was secured within an individual PVC-pipe apparatus (Fig. 1a) to ensure minimal movement within trials. A partition base (6' x 6') composed of PVC pipes and covered by a dark-colored tarp (8' by 10') was used to obscure the experimenters and supplies from the horses between trials (Fig. 1b). Three video cameras were placed at different locations within the test area to capture the horses' movements and choice within a trial. A Canon Vixia HF R700 was placed behind the right side of the horse, a Go-Pro HERO Session was attached to the experimenter and recorded the front of the horse, and an Iphone-7 was placed on the side of the bucket and recorded from the left of the horse. All personnel (experimenters, handlers and bystanders) wore mirrored sunglasses to avoid visual cues, such as eye movements towards a baited container, and both experimenters wore neutral-colored clothing to maintain uniformity and avoid responses due to unintended factors (Smith et al., 2018).

Figure 1. Photographs of experimental materials, including **a)** the PVC-pipe apparatus and bucket and **b)** the PVC-pipe partition and tarp.

Procedure

The experiment was conducted from March through June 2018. All horses were led directly from their individual stalls to an outdoor paddock for training, test and control trials. The lead experimenter (experimenter #1) was wholly responsible for conducting training and control trials, yet, to ensure equal socialization, both experimenters fed and petted each horse each time they arrived in the test area (Cook et al., 2014). No horse was involved in sessions for longer than 20 minutes, consecutively in any session. To minimize confounds, all handlers and experimenters were female. Based on the attention and temperament of the horses, handlers were swapped as needed. Experimental phases were counterbalanced and a random number generator was used to randomize the side for bucket baiting (left or right), controls (pointing or control and left or right) and experimenter identity (accurate or inaccurate). All randomizations appear in Table 2.

Table 2. *Experimental randomizations for use during test trials and controls*

Horse # and Name	Phase Order	Baited Order First Phase	Baited Order Second Phase	Control: Type of Trial and Location	Accurate Experimenter
#1, Buddha	Phase 1 first	LR, LR, RL	RL, RL, LR	AL, AL, AR, CR, AL, AR, CR, CL, AR, CL, AR, CL, CR, CL, AL, AR, CR, AL, CL, AR,CL, AL, CR, CR	Experimenter #1
#2, Captain	Phase 1 first	RL, LR, RL	RL, LR, LR	AR, CR, AL, CR, AR, AR, AR, AL, CL, AL, AR, CL, CL, CR, CR, CR, CL, AL, CL, AL, AL, CL, AR, CR	Experimenter #2
#3, Journey	Phase 1 first	LR, RL, RL	RL, RL, LR	CR, AL, CR, CL, CL,AR, CR, AL, AR, CL, CR, CL, CL, AR, AL, AR, AL, CR, AL, CL, AR, CR, AL, AR	Experimenter #1
#4, Codex	Phase 1 first	LR, RL, RL	RL, LR, LR	CR, AR, CR, AL, CL, CR AL, AL, AR, AR, CR, CR CR, AL, CL, AR, CL, CL CL, AR, CL, AR, AL, AL	Experimenter #2
#5, Jack	Phase 2 first	LR, LR, RL	LR, LR, RL	CR, CR, AL, AR, CR, CR AR, CR, AL, AL, AR, AL CL, CL, AL, AR, AL, CL AR, CL, CL, CR, CL AR	Experimenter #2
#6, Irene	Phase 2 first	LR, RL, LR	LR, LR, RL	AL, AR, CR, AL, CL, AR, CL, CL, CR, CL, AR, CL, AL, AR, AL, AR, CR, CR, CR, AR, AL, CL, AL, CR	Experimenter #2
#7, Sporty	Phase 2 first	RL, LR, LR	RL, LR, RL	AR, AR, CL, CL, CR, AL, AL, AL, AL, CL, CL, CR, AR, AL, CL, AR, AL, CR, AR, CR, CL, CR, AR, CR	Experimenter #1
#8, Bea	Phase 2 first	RL, LR, LR	LR, RL, LR	AR, CL, CL, CL, AL, AR, AR, AL, AL, CL, CL, AR, AR, AL, CR, CR, CR, AR, AL, CR, CR, CL, AL, CR	Experimenter #1

Horses received randomized labels of 1-8 that determined counterbalanced phase orders. The events of phase one and phase two were firm, yet the baited bucket was randomized for each horse. For phase one and phase two, the accurate and inaccurate experimenter was randomized for each horse and remained consistent throughout all 12 trials of phases one and two. Controls were split into accurate pointing trials and control trials, where the order and baited location was randomized for each horse. AL = accurate left (pointing trial). CR = control right (no-pointing trial).

All trials were conducted in the same barn aisle at the Ramapo Equestrian Center. Based

on temperament, varied amounts of time were allotted for individual horses to investigate the

experimental set-up. The partition remained in the same spot throughout all trials of training,

testing and controls in order to desensitize the horses to the unfamiliar item. The test area (Fig. 3)

aisle was bordered by individual horse stalls, however, bystander horses were not included in the

study. The partition was 1.9 meters wide and was placed in the middle of the 4.2-meter test aisle

so that the distance between the partition and the wall was approximately 1.1 meters. The

midpoint of the partition was 0.9 meters. Buckets were spaced 43.2 centimeters from either side

of the midpoint and 87.6 centimeters apart from one another. The buckets were positioned 33.0

centimeters away from the foot of the partition. A mark was made at the midpoint of the partition

and 50.8 centimeters were triangulated from the inner, back corner of each bucket to determine

where the experimenter would stand relative to the buckets and partition. The experimenter stood

with her back heels in line with the front of the partition legs. A four-meter chalk path was

marked from the release point to guide the horses across the test area. Buckets were spaced 25.4

cm away from the fourth meter mark.
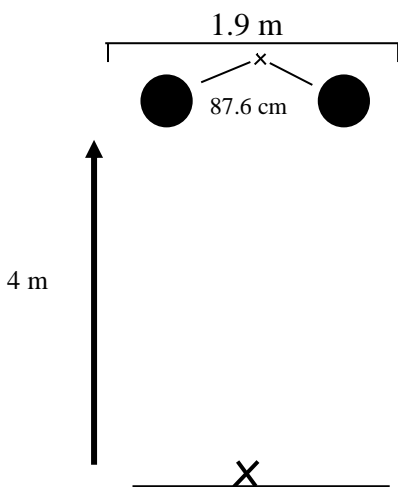
Figure 2. Schematic diagram of experimental test area including the placement of materials and personnel and key measurements that remained uniform throughout the study.

During all pointing trials, the experimenter stood between the buckets and followed a

three-step signaling process (Fig. 3). The beginning of each trial varied based on the stage of the

experiment, yet within all test trials, the experimenter called the horse by name and waited for it

to look at the experimenter and approach. Once the horse was two meters away from the release

point, the experimenter would point directly at the horse while orienting the body upright and

straight ahead (Fig. 3a). When the horse was three meters away from the release point, the

experimenter would display a sustained proximal and ipsilateral point cue towards one of the two

buckets. This pointing cue was purposefully exaggerated due to horses' proposed reliance on

stimulus enhancement (Proops et al., 2010), and involved rotating the arm, body and eyes in the

direction of the bucket (Fig. 3b) and pointing downward (Fig. 3c). During this signal, the

experimenter leaned closely towards the bucket, and maintained a submissive posture believed to

encourage horse approach behavior (Smith et al., 2018). Handlers always stood to the left of the

horse, yet between each trial they were alternatively led to the left or right, and back around to

the release point. Throughout each trial, the handler was instructed to maintain a loose grip on

the horse's lead rope, stay behind the horse and only redirect them if they veered off of the

designated path. A duct tape path that spanned four meters was placed to the left of the release

point, and the handler was instructed to follow this path and keep their eyes and head down for

the duration of each trial. Verbal or physical cues within a trial were prohibited, yet after correct

choices only, the experimenter and handler provided verbal reinforcement. Correct choices were

rewarded with one or more small carrot pieces. Any behavior that resulted in a horse failing to

reach the four-meter mark or approach before being called was categorized as a 'false start' and

resulted in an immediate repetition of that trial. Between each trial the handler was instructed to

verbally reinforce the horse and, if necessary, refocus their attention for the next trial. The use of

verbal reinforcements between trials, and test trial formation were adapted from Proops et al.

(2010).

Figure 3. Example of bucket baiting during a trial. This three-step process was standardized across both experimenters, and used during training, test and control trials where a point was applicable.

Decisions regarding exclusion from the study or attempted retraining were determined for individual horses on a case-by case basis. Minimal adjustments to accommodate individual horse needs were permitted (e.g. hand-feeding, extra training sessions, re-focusing exercises). Horses were typically excluded based on temperamental issues that precluded them from progressing through training and tests. If horses within experimental trials displayed significant side biases or repeated failure to respond at all within the experimental trials, the data were discarded and the horse was given a break of one week to attempt retraining. This involved a shortened version of training (Table 3), where T2 consisted of a minimum of 6 trials (if accuracy was 100 percent) and a maximum of 12 trials. If issues remained following retraining, the horse was excluded from the study.

Training

Prior to the experiment, all horses completed three training sets that appeared in the same order and increased in difficulty (Table 3). Training sets were designed to incrementally familiarize the horses with human pointing cues within an object-choice task. Each horse was required to successfully complete three training sets and continual refreshers throughout the

experiment. Based on the attention of the horses, training was either completed consecutively, or with a break between sets. The training protocol is listed in Table 3.

Training set #1 (T1). Three open-baited trials with one bucket were conducted. For all T1 trials, the experimenter stood at the midpoint of the partition and directly behind the single bucket. The experimenter dropped carrots into the bucket as the horse approached. There was no time limit on retrieving the carrots, and additional trials were conducted as needed. This protocol was designed for the horse to create an association between the bucket, food and experimenter.

Training set #2 (T2). Six open-baited trials with two buckets were conducted. For all T2 trials, the horse watched the experimenter 1) drop carrots into one open bucket and then 2) provide a sustained proximal pointing cue (Fig. 3). The horses were permitted to approach and investigate both buckets, however, after reaching the buckets, they were allotted a maximum of ten seconds to retrieve the food.

Training set #3 (T3). Between 12 and 48 closed-baited trials with two buckets were conducted. In order to continue to the experimental test trials, horses needed to reach a criterion of 80 percent accuracy across two consecutive sets of six trials each. Before and after each trial, buckets were moved behind the partition and food was placed into one undisclosed bucket. The horses, handlers and bystanders were blind to the location of the food. For all T3 trials the experimenter provided a sustained proximal pointing cue towards the bucket containing carrots. A choice was only counted as 'complete' if the horse grazed its whiskers to the bucket. In the case of horses without whiskers, a choice was only counted following direct contact. Once the horse approached the buckets, it was required to make a choice within ten seconds. After each trial, the bucket was opened. If the horse made the correct choice, it had access to the carrots, however, if the horse made the incorrect choice it was shown an empty bucket.

Table 3. *Experimental training protocol*

Training Phase	Number of Buckets	Baiting	Pointing	Number of Trials	Conditions
T1	1	Open	None	3	Freely investigate
T2*	2	Open	Yes	12	Freely investigate, 10 seconds
T3	2	Closed	Yes	12-24	First choice, 10 seconds

All horses were required to successfully complete training at or above criteria level (80%) in T3. Each training phase increased in difficulty, where T3 mimicked experimental test trials. *If horses had to be retrained due to side biases or failure to respond during test trials, T2 consisted of a minimum of 6 trials with 100% accuracy or 12 trials. Otherwise, retraining protocol was identical to training.

Refresher. When horses were not used for more than four days, a refresher was conducted based on their individual progress. For the horses that completed T1 and T2 of training, a refresher of one trial of T1 and three trials of T2 was conducted prior to beginning T3. For the horses that had completed training and were at various stages of the experiment or control, the refresher included one trial of T1, three trials of T2 and three trials of T3. During T3 refreshers, 2/3 accuracy was required to continue with the experiment. If this was not achieved, a short break was provided before T3 was reattempted. When time allowed, horses were refreshed weekly, regardless of their participation in the experiment that week.

Bucket Refresher. If, during the refresher for T3, we noticed that individual horses were no longer making direct contact with the bucket (via whiskers or otherwise), we implemented a bucket refresher, "T2.5". This set involved trials with two closed-baited buckets, yet in addition to the food hidden within the bucket—a combination of carrots and a high-value food reward, "Applezz N Oats"—the experimenter also placed the latter food on the lid. When the experimenter provided a sustained proximal pointing cue to the bucket, the horse was incentivized to make direct contact with the lid, and upon contact, the bucket was opened to

reveal the treats hidden within. A criterion of 2/3 correct choices was required for horses to

reattempt T3.

General Test Procedure

At least one week was placed between phase one, phase two and phase three (control).

Each horse was exposed to two experimenters that remained consistent in their roles for all trials.

One experimenter was referred to as the "inaccurate experimenter", and they provided a

predetermined quantity of inaccurate and accurate pointing trials for sets A through E. The other

experimenter was referred to as the "accurate experimenter", and conducted set F, where they

provided only accurate pointing cues. Accurate pointing cues were defined as trials where the

experimenter pointed towards a baited bucket, whereas inaccurate pointing cues were defined as

trials where the experimenter pointed towards an unbaited bucket. The handler and any unrelated

bystanders were blind to the goal of individual trials (accurate versus inaccurate), and the

location of food within a trial. After reaching the four-meter mark, horses were required to make

a choice within 10 seconds. Regardless of the choice made, the bucket was opened, and the horse

either had access to the carrots, or was shown an empty bucket. Buckets were moved behind the

partition between each closed-baited trial to ensure handlers, horses and bystanders were blind to

the baiting. Similarly to training set #3, all trials required direct contact with the buckets, either

through their whiskers or head. These phases were adapted from Takaoka et al. (2015). The

experimental test trial and control protocol is listed in Table 4.

Phase One. This phase consisted of six total trials, where the sets were referred to as A,

B, and C, respectively. A) For the first two trials, the experimenter displayed a sustained

proximal pointing cue towards the bucket containing the carrots with both buckets closed-baited.

B) For the next two trials, open buckets were placed in the test area with their lids set behind

them. The experimenter clapped to get the horse's attention, dropped carrots into one bucket, tilted both buckets forward to reveal their contents and closed them. The experimenter then called the horse's name, and displayed a sustained proximal pointing cue towards the bucket without food. Horses were required to avoid experimenter cues in order to access the food reward. C) For the last two trials, the experimenter displayed a sustained proximal pointing cue towards the bucket containing the carrots with both buckets closed-baited.

Phase Two. This phase consisted of six trials, where the sets were referred to as D, E, and F, respectively. The purpose of this phase was to ensure that the potential results in the latter portion of phase one—such as reduced response rates in set C—were not due to fatigue or diminishing motivation (Takaoka et al., 2015). Introducing another six trials with three sets, where sets D and E were identical to sets A and B, allowed us to use set F to evaluate the influence of experimenter identity on subsequent choices. During set F, a new experimenter appeared and displayed an accurate sustained proximal pointing cue for two closed-baited trials.

Phase Three (control). This phase was designed to evaluate the influence of olfactory cues on choices, and provide insight into potential side-biases across conditions. After phase one and phase two were completed, 24 closed-baited trials were conducted. Half of these trials were controls (in which no pointing cue was provided but all other aspects of the trial were identical to set A above), and half of these were accurate pointing trials (identical to set A). These 24 trials appeared in a randomized order (Table 2) and were designed to maintain motivation and avoid frustration or fatigue that may have resulted from repeated failure to access the food. Twelve accurate trials gave the horses an opportunity to respond correctly based on pointing cues, rather than chance. During the 12 accurate trials, a sustained proximal pointing cue was directed towards one of two closed buckets. During the 12 control trials, the experimenter kept their eyes

and body facing forward while providing no visual cues (Plotnik et al., 2013; Smet & Byrne,

2013). If carrots no longer motivated the horse, a higher value treat, Applezz 'N Oats, was used

in conjunction with carrots. If horses seemed fatigued, unmotivated or distracted, a short break

was imposed after the first 12 trials.

Table 4. *Conditions for experimental phases and controls*

Phase	Open/Closed	Accurate/ Inaccurate	Pointing/ No Pointing	Number of Trials	Experimenter
1A	Closed	Accurate	Pointing	2	A
1B	Open during baiting, closed immediately after	Inaccurate	Pointing	2	A
1C	Closed	Accurate	Pointing	2	A
BREAK	--	--	--	--	
2D	Closed	Accurate	Pointing	2	A
2E	Open during baiting, closed immediately after	Inaccurate	Pointing	2	A
2F	Closed	Accurate	Pointing	2	B
BREAK	--	--	--	--	
3 (Control)	Closed	Accurate	12 pointing, 12 non-pointing	24	--

All trials involved two buckets, and required the experimenter to call the horse's name and secure its attention before proceeding with the trial. (A= inaccurate experimenter provides inaccurate or accurate cues; B= new, accurate experimenter provides accurate cues). All control trials were conducted by the lead experimenter.

Analysis

Obedience was measured based on the responses of the horses in each set. If a horse

followed a pointing cue twice (100%) within a set, they were considered obedient. If a horse

followed a pointing cue fewer than two times (50% or less) within a set, they were counted as

non-obedient (Takaoka et al., 2015). For each individual set (A through F) chance was set at

0.25. This was based on the four possible outcomes that could occur within a set: following a cue

in both trials, following a cue in the first trial only, following a cue in the second trial only or

following the cue in neither trial. Obedience was used to evaluate the influence of experimenter

identity (accurate versus inaccurate) on the choices and behaviors of subjects.

The results from each trial were transcribed by an experimenter at the time of data

collection, and included: baited bucket order, handler and choice. Choices were either written as

"Yes", "No" or "No Choice (NC)". A trial was coded as "Yes" if the horse followed the pointing

cue, regardless of whether the cue was accurate or inaccurate. NC trials were included in the

"No" category, but were informative when analyzing the impact of experimenter inaccuracy on

horse behavior. After each field site visit, all records were corroborated by video evidence

collected from the three cameras. At the end of the experiment, latency to approach the buckets

was calculated for each individual test trial. The format for coding was standardized such that the

approach timer began when the horse lifted its foot off the ground and ended once a choice was

made. Trials automatically ended after ten seconds of inactivity at the four-meter mark,

regardless of whether or not the horse made a choice. This resulted in some longer latencies

accompanied by "NC" as referenced above.

Changes in the proportion of obedience were compared across phase sets (e.g. A, B, C,

D, E, F) using a McNemar's test. Obedience within each set was measured using a binomial test.

All data were analyzed as a whole ($n = 8$) and between phases ($n = 4$ and $n = 4$) to evaluate the

role of order effects on obedience between and within sets. Latency to approach the buckets was

evaluated using a Friedman's ANOVA for phase one (A, B and C) and phase two (D, E and F).

Further analyses of the change between trial latencies were evaluated using a Wilcoxon signed-

ranks test. Controls were analyzed using a Wilcoxon signed-ranks test that compared the success

in finding food on accurate trials to success in control trials. A second individual that was

uninvolved in the experiment coded 25% of test trials. Inter-rater reliability scores on latency ($r =$ 0.93) and correct choice (96%) were high. All analyses were performed by hand using calculations from Siegel & Castellan (1988) and IBM SPSS version 24.0 for Macintosh (IBM Corp. Armonk, NY, U.S.A.)

Results

The number of trials required for each individual horse to successfully complete T3 is listed in table 5 (mean = 16.6, SD = 11.00). All choices across the 12 trials of phase one and phase two are listed in Table 6a, and further categorized in Table 6b to include all "no" responses versus all "no choice" responses. All permutations of individual horse trials, based on phase order and identity of the experimenters (e.g. experimenter #1 as inaccurate and experimenter #2 as accurate, and vice versa), and their subsequent choices are listed in table 7.

Table 5. *Number of trials required for successful completion of training session #3 (T3)*

Horse	Number of trials (T3)
Buddha	12
Captain	46
Journey	12 (12)
Codex	12
Jack	12
Irene	12
Sporty	12
Bea	12 (24)

A minimum of 12 trials were required for T3 completion. Horses with two values (e.g. Journey and Bea) required repeat training sessions due to technological or side bias issues. Second training sessions are listed in parentheses.

Table 6a. *List of horses' choices across all 12 trials of phases one and two*

Horse	Phase											
	1A1	1A2	1B1	1B2	1C1	1C2	2D1	2D2	2E1	2E2	2F1	2F2
Buddha	Y	Y	N*	Y	Y	N*	Y	Y	N*	Y	N*	N*
Captain	Y	Y	Y	Y	Y	Y	Y	N*	Y	Y	Y	Y
Journey	Y	Y	Y	Y	N*	Y	N*	Y	N	Y	Y	Y
Codex	Y	Y	Y	Y	Y	Y	N	N	Y	N*	Y	Y
Jack	N	N	N*	Y	N	N*	Y	Y	N	Y	Y	N*
Irene	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N
Sporty	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N
Bea	Y	Y	Y	N	N	N	Y	Y	Y	Y	Y	Y

Y = followed cue. N = avoided cue. N* = no-choice. Trials were split into individual cells, yet obedience was determined by the combined results from each set.

Table 6b. *Distribution of "No" choices during experimental trials*

	B	C	E	F	Total
No	2	4	2	2	10
No Choice	2	3	2	3	10
Total	4	7	4	5	20

Half of the trials in which "no" was coded, horses failed to make a choice within the allotted time, whereas the other half of "no" trials, horses approached the bucket not associated with the pointing cue.

Table 7. *Experimental conditions based on phase order and experimenter identity*

	LA		LB		LC		LD		LE		KF	
	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2
Captain	Y	Y	Y	Y	Y	Y	Y	N*	Y	Y	Y	Y
Codex	Y	Y	Y	Y	Y	Y	N	N	Y	N*	Y	Y
	LD		LE		KF		LA		LB		LC	
	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2
Jack	Y	Y	N	Y	Y	N*	N	N	N*	Y	N	N*
Irene	Y	Y	Y	Y	Y	N	Y	Y	Y	N	Y	Y
	KA		KB		KC		KD		KE		LF	
	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2
Buddha	Y	Y	N*	Y	Y	N*	Y	Y	N*	Y	N*	N*
Journey	Y	Y	Y	Y	N*	Y	N*	Y	N	Y	Y	Y
	KD		KE		LF		KA		KB		KC	
	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2	Trial #1	Trial #2
Sporty	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y
Bea	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N

Horses received one of four possible test trial permutations. Horses either received phase one first and experimenter #1 as inaccurate, phase two first and experimenter #1 as inaccurate, phase one first and experimenter #2 as inaccurate or phase two first and experimenter #2 as inaccurate. L = experimenter #1. K = experimenter #2. Y = followed cue. N = avoided cue. N* = no-choice.

Using McNemar's test, this study first evaluated the change in obedience levels between sets of phases one and two. Following a one-week or longer break separating phases one and two, horse obedience levels did not significantly differ between sets A and D (McNemar's test: p = 0.63; Fig. 4). After the inaccurate cues that occurred in set B, horses displayed a trend for reduced obedience from set A to set C (McNemar's test: p = 0.06; Fig. 4), yet there was no difference in obedience levels between sets D and F (the latter of which involved the introduction of a new experimenter; McNemar's test: p = 1.00; Fig. 4). Overall, experimenter

identity did not cause a significant difference in horse obedience levels between set C and set F (McNemar's test: p = 0.50; Fig. 4). Horses that received experimenter #2 as the inaccurate experimenter showed a trend for reduced obedience between set A and C (McNemar's test: p = 0.06; Table 8a), whereas subjects that received experimenter #1 as the inaccurate experimenter did not show a trend for reduced obedience between step A and C (McNemar's test: p = 0.50; Table 8b). However, all horses were obedient for their first set of trials, irrespective of the individual acting as experimenter #1 or experimenter #2 (see Table 7). The order of experimental test trials (e.g. horses that received phase one prior to phase two or vice versa) did not reveal any significant changes in obedience between set A and D (McNemar's test: phase one first, p = 0.25; phase two first, p = 1.00), set A and C (McNemar's test: phase one first, p = 0.25; phase two first, p = 0.25), set D and F (McNemar's test: phase one first, p = 0.63; phase two first, p = 0.25) or set C and F (McNemar's test: phase one first, p = 0.50; phase two first, p = 0.50).
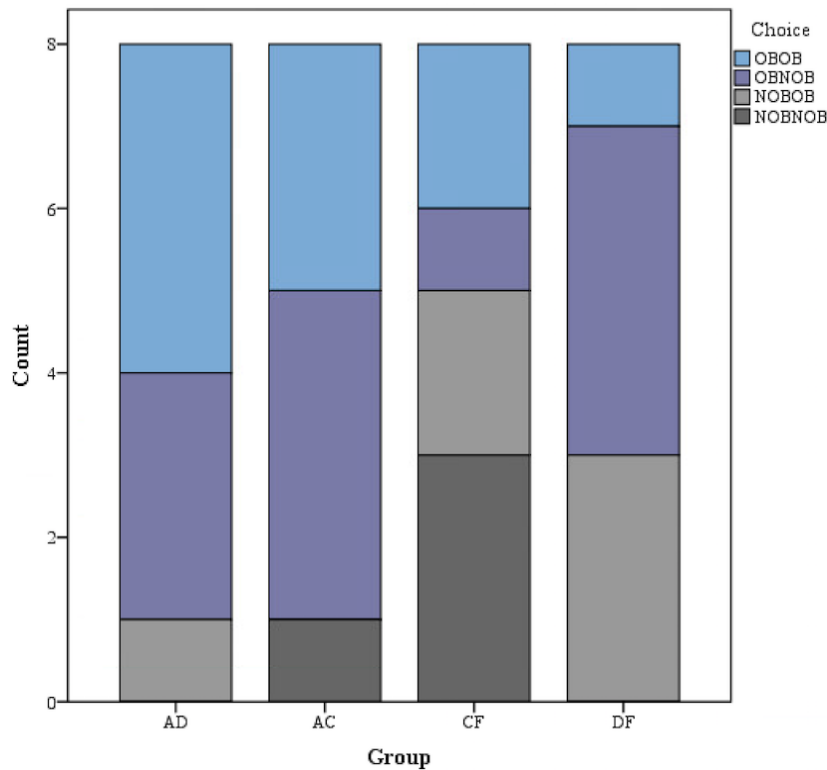
Figure 4. Proportion of changed obedience between experimental sets. Each column represents the proportion of change in obedience across two sets (A and D, A and C, C and F, and D and F). OB = obedient, NOB = non-obedient. All columns are based on the entire sample (*n* = 8).

Table 8. *Obedience based on familiarity*

a)

		C	
		OB	NOB
A	OB	0	4
	NOB	0	0

b)

		C	
		OB	NOB
A	OB	3	0
	NOB	0	1

The influence of experimenter familiarity on the proportion of changed obedience between steps A and C where a) the inaccurate experimenter is less familiar and b) the inaccurate experimenter is more familiar. OB = obedient. NOB = non-obedient. Table 8a and 8b are based on half of the sample *(n = 4).*

This study used binomial tests to evaluate the obedience of horses (as a whole and based on their phase order), relative to chance levels (0.25). Horses were more obedient than expected by chance in set A (Binomial test: $p < 0.01$) and set D (Binomial test: $p = 0.03$), however, when evaluated based on their randomly counterbalanced orders, all horses were 100% obedient (Binomial test: $p < 0.01$) in their respective first sets (A or D). Of those who received phase one first, obedience was above chance during set A (Binomial test: $p < 0.01$), set B (Binomial test: $p = 0.05$) and set F (Binomial test: $p = 0.05$), whereas those who received phase two first displayed above chance obedience during set A (Binomial test $p = 0.05$), set D (Binomial test: $p < 0.01$) and set E (Binomial test: $p = 0.05$). Binomial calculations are listed in table 9.

Table 9. *Binomial calculations of obedience proportions*

	Entire Sample (*N = 8*)	Phase One First (*N = 4*)	Phase Two First (*N = 4*)
A	7/8 OB, 87.5% (p < 0.01)*	4/4 OB, 100% (p < 0.01)*	3/4 OB, 75% (p = 0.05)*
B	4/8 OB, 50% (p = 0.11)	3/4 OB, 75% (p = 0.05)*	1/4 OB, 25% (p = 0.68)
C	3/8 OB, 37.5% (p = 0.32)	2/4 OB, 50% (p = 0.26)	1/4 OB, 25% (p = 0.68)
D	5/8 OB, 62.5% (p = 0.03)*	1/4 OB, 25% (p = 0.68)	4/4 OB, 100% (p < 0.01)*
E	4/8 OB, 50% (p = 0.11)	1/4 OB, 25% (p = 0.68)	3/4 OB, 75% (p = 0.05)*
F	4/8 OB, 50% (p = 0.11)	3/4 OB, 75% (p = 0.05)*	1/4 OB, 25% (p = 0.68)

Rows correspond to obedience proportions and significances of each experimental phase. Columns indicate the three portions of binomial data analysis: the entire sample (*n = 8*), phase one first subjects (*n = 4*) and phase two first subjects (*n = 4*). *significant value at α = .05.

To assess side biases, a Heterogeneity G-test was run for all 8 horses comparing the number of left bucket choices to right bucket choices. As a group, the horses did not show a side bias for either the left or right bucket across all 12 test trials (Heterogeneity G-test, Gh = 5.62, df = 7, p = 0.58, Gp = 0.42, df = 1, p = 0.51). To assess the influence of non-social cues such as olfaction on the horses' choices, success on the 12, no-pointing control trials was compared to success on the 12 accurate, pointing trials across the group of horses; a significant difference was found between the horses' performance on the two types of trials (Wilcoxon Signed-Rank test: W = 0, p < 0.05).

Latencies to choose a bucket across test phases and trials were measured to evaluate the influence of inaccurate cueing and experimenter identity on subsequent response rates. Horses did not show significant differences in their latencies to choose a bucket across the sets of phase

one (Friedman test: $\chi^2(2) = 1.31$, $p = 0.52$) or phase two (Friedman test: $\chi^2(2) = 0.75$, $p = 0.69$). The difference in latencies between the first trial of set B (B1) and the second trial of set B (B2) were not significantly different than the latencies between the first trial of set A (A1) and the second trial of set A (A2) (Wilcoxon Signed-Rank Test: $W = 10$, $p > 0.05$). Similarly, the difference in latencies between D1 and D2 were not significantly different than the latencies between E1 and E2 (Wilcoxon Signed-Rank Test: $W = 14$, $p > 0.05$). Differences between latencies remained non-significant when order effects were accounted for, and respective first sets (A or D) were compared to respective second sets (B or E) (Wilcoxon Signed-Rank Test: $W = 8$, $p > 0.05$). When the difference in latency between set B and set C was compared to the difference in latency between set E and set F, no significant difference was found (Wilcoxon Signed-Rank Test: $W = 10.5$, $p > 0.05$).

Horses did not show any indication of a win-stay, lose-shift learning strategy. Of those who were obedient in set A, there was no significant difference in their obedience between set A and set B (McNemar's test: $p = 0.13$). Similarly, those that were obedient in set D, did not significantly differ in their obedience between set D and set E (McNemar's test: $p = 0.25$). There is no indication that horses were less obedient after deception in general. Trials before (set A and set D) and after (set C and set F) the intervention were pooled, and revealed no significant difference (Wilcoxon Signed-Rank test: $W = 7$, $p > 0.05$).

Discussion

This study evaluated the influence of inaccurate pointing cues on horse behavior in an object-choice task. Based on the protocol of Takaoka et al. (2015), this study designed a two-phase object-choice task, where phase one evaluated the role of inaccurate experimenter pointing cues on subsequent horse responses, and phase two evaluated the effect of a new experimenter

(with whom the buckets were accurately indicated) on horse behavior. In addition, this design controlled for the possibility that horses' behavior changed over the course of a phase because of fatigue and not because of the condition or experimenter. Consistent with the work of Takaoka et al. (2015) where dogs were shown to preferentially avoid signals from an inaccurate experimenter, our results indicate that domestic horses show a trend for reduced obedience after receiving inaccurate pointing cues (Fig. 4), yet, additionally, seem to preferentially apply these behaviors based on their familiarity with the experimenter (Table 8). Specifically, horses only inhibited cue-following when the experimenter was less familiar, such as in the case of experimenter #2, with whom the horses had no training experience prior to the test trials. In contrast, when the inaccurate experimenter was more familiar, such as in the case of experimenter #1, with whom all training trials were conducted, horses did not inhibit responses between sets A and C.

In contrast to the trend of reduced obedience between sets A and C, there was no significant difference in obedience between sets D and F. This, again, further supports the notion that horses adapt their responses based on the identity of the experimenter—specifically, their familiarity—and exhibit discriminatory capabilities when evaluating choices within a cognitive paradigm. However, it is critical to note that there was no significant difference in the horses' obedience between sets C (37.5%) and F (50% - Table 9), which may have been influenced by the recency of inaccurate pointing cues from sets B and E, respectively. Overall, these results make sense in light of previous studies on horse behavioral flexibility (Krueger et al., 2011; Proops & McComb, 2010; Ringhofer & Yamamoto, 2017; Smith et al., 2018), where horses seem to apply knowledge, body cues and attentional states of experimenters to better succeed within problem-solving tasks.

Our results also indicate that horses may be capable of memory retention, and utilize this

information to succeed in a cognitive task. The encoding specificity theory posits that the

retrieval of memory is dependent on the context of storage (Tulving & Thomson, 1973), and it is

likely that horses' memory for experimental protocol is strengthened by the presence of

consistent human and environmental conditions. Despite limited previous research on the scope

of horse memory retention (Brubaker & Udell, 2016), horses have been shown to exhibit long-

term and short-term memory in a range of cognitive tasks (Hanggi & Ingersoll, 2009; Krueger et

al., 2011), and in respect to human vocal recognition (Proops & McComb, 2012). Similarly, in

this experiment, most horses responded to pointing cues during their first set of inaccurate

pointing (B or E) yet after a one-week break was imposed between phases, most no longer

responded to inaccurate pointing cues (Table 9). In these conditions, horses either ignored the

pointing cue entirely and made no choice, or approached the opposite bucket. The horses'

tendency to follow the first set of inaccurate pointing cues, likely due to their training—since all

previous training and test trials employed accurate pointing cues—is similar to the responses of

domestic dogs (Kundey et al., 2010; Takaoka et al., 2015), where they exhibit difficulty avoiding

human signals, even when they are overtly misleading. This suggests that horses may have a

similar tendency to attend to human cues (Kundey et al., 2010), yet subsequently adapt them

based on their familiarity with the experimenter (Cook et al., 2014).

Furthermore, the counterbalanced order in which horses received the two experimental

phases likely affected behavioral responses. Our results showed that only horses that received

phase one first (P1 horses) followed experimenter pointing cues from the new, accurate

experimenter in set F (Table 9). This is not surprising, given that P1 horses had substantially

more opportunities to develop a negative association with the inaccurate experimenter (Table 4).

In contrast to horses that received phase two first (P2 horses) and thus had only four trials (sets D and E) prior to set F, P1 horses experienced 10 trials (sets A through E) prior to set F. Although obedience levels across the entire sample ($n = 8$) were not different between sets A and D, our results also showed P1 horses did not follow experimenter pointing cues in set D, while P2 horses successfully followed experimenter pointing in set A. P2 horse responses may have been influenced by set F, which was their most recent test set from the previous week. It is possible that these horses may have remembered the previous positive experience with a new experimenter, while, in contrast, P1 horses had most recently experienced an experimenter in set C who had been inaccurate in set B. This relates to our above findings, where horses showed some evidence of memory retention based on positive or negative experiences within the experiment.

Unlike the findings of Takaoka et al. (2015) where dogs were significantly slower to approach the inaccurate experimenter after they had provided a misleading cue, our results did not find any significant differences between latencies across any of the experimental phases. These results may be due to the experimental design, which required that horses make a choice between the two buckets within 10 seconds of approaching the experimenter. This resulted in some trials ending exactly 10 seconds after the horses' arrival to the buckets, due to their inactivity. Additionally, due to the horses' extensive training and human socialization, it is likely that they intentionally matched the pace of the handler who walked alongside them.

As indicated through previous research (Krueger et al., 2011; Proops et al., 2010) and supported by our results, horses respond to stimulus enhancement cues within an object-choice task. Similarly to Proops et al. (2010), at times, horses in this experiment approached the experimenter's hand, prior to selecting a bucket. Within our study, some horses proficiently

followed pointing cues after extensive training, while others (either due to fear or disinterest) were unable to follow the cue, and were thus excluded from the study. This supports the prevailing notion that many factors, including genetics, environment, ontogeny and domestication, likely contribute to domesticated animal behavior (Hare et al., 2010; Hausberger et al., 2004; Hausberger et al., 2008; Kaminski et al., 2005; Proops et al., 2010; Ringhofer & Yamamoto, 2017)

Interestingly, this study revealed temperament differences between horses, where some horses, such as Captain (horse #2) and Sporty (horse #7), were consistently obedient across sets B and E. Other horses, such as Buddha (horse #1) and Codex (horse #4) showed behavioral signs of trepidation following inaccurate pointing cues. Specifically, in set E, trial 1, Buddha approached the experimenter, and oscillated his head back and forth between the two buckets until the trial was terminated at 10 seconds. Similarly, Codex followed a misleading pointing cue during the first trial of set E, yet during the second trial of set E hovered above the bucket until the trial was terminated at 10 seconds. Similarly to Takaoka et al. (2015), this study included a limited number of trials (and specifically only allotted two trials for sets B and E) to avoid horses learning a "rule" that approaching a bucket that is not cued results in a food reward. According to Petter et al. (2009), dogs require multiple trials to learn these types of rules, yet, future studies on horse cognition should more closely evaluate the emergence of rule-following within cognitive tasks (McKinley & Sambrook, 2000; Proops & McComb, 2010).

Due to a small sample size, especially in the case of counterbalanced analyses ($n = 4$ for each sample) it is important to view these results conservatively. Results should be viewed in light of unavoidable variations in test conditions, handling, experimenters, and diet and attentional differences. Although all handlers were trained identically, individual variations exist

between handling techniques (e.g. walking pace, lead length and horse familiarity). Although both experimenters (experimenter #1 and experimenter #2) socialized with all subjects, the lead experimenter (experimenter #1) had more exposure to each horse, due to her role in training and controls. In contrast to open-baited training trials, where carrots were dropped into a bucket as the horse approached the experimenter, inaccurate pointing cue sets (B and E) may have been difficult for the horse to see, since baiting occurred while the horse stood at the release point, 4-meters away from the experimenter. However, we expected that by the second trial, they would have enough doubt to avoid experimenter cues, which turned out to be the case. All horses were led from the horse's left side, which could have affected the horse's preference for a given side when choosing a bucket. The data, however, show that the horses did not demonstrate side biases. Different attention spans within horses were noticed, where some required refocusing exercises between trials and had difficulty maintaining their focus throughout the experiment. Since the experiment was conducted over the course of three months (and between seasonal changes), horses may have been motivationally affected by weather differences or excessive heat. Lastly, ponies (Irene, horse #6 and Sporty, horse #7) had a more restrictive diet at this particular facility, which may have affected their motivation within the task. Due to experimenter errors and technological issues, phase one for Journey (horse #3) and phase two for Jack (horse #5) were repeated. Bea (horse #8) required retraining after an extreme left side bias (100%) was revealed during phase two of the experiment and persisted through a week-long break and refresher. This original data were discarded, and after completing the abridged version of training (Table 3), her subsequent trials revealed no discernable side biases, and thus were included in the final analysis.

These results contribute to the existing field of domesticated animal cognition, by revealing the behavioral flexibility of domestic horses within an object-choice task. The horses in this experiment showed evidence of preferential attendance towards relevant cues, and possible indication of memory retention across experimental trials. These findings contribute to our understanding of domestic horses' higher cognitive processing in human-mediated tasks, and suggest similar plasticity to domestic dogs (Takaoka et al., 2015). Similarly to dogs (Hare & Tomasello, 1999), we do not yet fully understand how horses comprehend human cues. However, it is possible that our study, among other investigations of horse behavioral flexibility and attention to human knowledge states (Brubaker & Udell, 2016; Krueger et al., 2011; Proops & McComb, 2010; Ringhofer & Yamamoto, 2017) may contribute to evidence for theory of mind. Further investigations into the underlying processes of signal detection in horses are critical. Future studies may also investigate the role of inaccurate conspecific cueing—through facial and body movements (Maros et al, 2008; Wathan & McComb, 2014; Waring, 2002) – on behavioral choices in an object-choice task. Specifically, the familiarity of conspecifics (in regards to housing facility and familial relationships) could be an important variable when evaluating the role of inaccurate cueing on behavioral responses between horses. Success in this task not only required attention to experimental procedures and cueing, but also required that the horse maintained a level of motivation and patience. The consistent participation of horses in this experiment may be useful when selecting horses for therapeutic riding programs, based on their individual temperament and adherence to human cues.

Our results provide evidence of domestic horses' plasticity, based on experimenter familiarity and memory of previous inaccurate pointing trials, when using human pointing cues in an object-choice task. This is surprising in light of previous research which has pointed to

horses' lesser degree of development in responding to human cues (McKinley & Sambrook, 2000; Proops et al., 2010). In this study, horses initially responded to all accurate experimenter pointing cues, irrespective of the source and familiarity (Table 7), which indicated their instinctive response—likely due to a combination of domestication and socialization (see Hare et al., 2010; Hare & Tomasello, 2005; Udell et al., 2008)—to attend to human cues. However, the fact that after receiving inaccurate pointing cues, in sets B and E, horses only inhibited responses towards the less familiar experimenter, points to the memory retention and relationship development between humans and horses. When taken together, these results indicate that horses can respond to all pointing cues, yet, following inaccuracy, prioritize their "trust" based on the experimenter's identity over the experimenter's action. Furthermore, when viewed in light of their resistance to follow the second set of inaccurate pointing cues (B or E depending on which phase the horses received first) (Table 9), this research provides a critical step forward in understanding the behavioral flexibility of horses, specifically as demonstrated through their social relationships with humans.

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