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Original Research

Responses of Domestic Horses and Ponies to Single, Combined and Conflicting Visual and Auditory Cues

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ABSTRACT

Domestic horses and ponies communicate using visual and auditory signals. It has been reported that equines can respond to visual cues in object-choice tests, but utilization of auditory cues, alone or associated with visual cues, has not been investigated. Effect of equine breed type in object-choice selection is unknown. Using object-choice tests, we investigated the hypotheses that breed types (1) can use both visual and auditory human-given cues; (2) that performance is enhanced when both visual and auditory cues are presented together to signal a baited bucket, compared with when a cue is presented singly; (3) that latency to make a choice increases and choice is random, when auditory and visual cues conflict; and (4) that ponies outperform horses. Irrespective of breed type, subjects were equally successful at using single visual, auditory, and combined cues (proportion of correct choices: visual 0.63 ± 0.047 [$P = .004$], auditory 0.61 ± 0.045 [$P = .013$], combined 0.64 ± 0.054 [$P = .007$]). In contrast to our hypothesis, combining cues did not significantly improve the likelihood of correct choice. Ponies outperformed horses using visual cues ($P = .044$). In conflicting cue tests, 70% of subjects responded randomly; the remainder preferentially responded to visual cues. Our study showed that horses and ponies can respond with equal proficiency to both visual and auditory cues, alone and combined; however, ponies outperformed horses using visual cues. Our results may be used to improve relationships between humans and equines, as we demonstrated the importance of engaging both visual and auditory modalities.

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1. Introduction

Domestic equine breed types (horses and ponies) are highly sensitive to cues, such as body language and auditory cues [1]. In relationships with human, equine breed types must correctly interpret human-given cues to learn novel skills, express desirable behaviors, and avoid actions unwanted by humans. Training often involves teaching equine breed types to respond to human-given cues

through secondary conditioning, controlling behavior with auditory or visual signals [2]. It is possible to evaluate animals' ability to respond, and understand, human-given cues by using object-choice tests [3]. However, such tests have not been widely used to assess the capacity of domestic equine breed types to perceive, respond, and interact with human-given cues. The few object-choice tests conducted with equine breed types have been confined to visual cues [4–7]. The capacity of equine breed types to respond to either auditory cues presented alone or simultaneous visual and auditory cues (combined cues) has not been investigated. The impact of presenting two cues that do not direct the animal to the same outcome (conflicting cues) may reduce the rate of correct responses,

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because in equine breed types, learning has previously been considered to be impaired by conflicting cues [8].

This study aimed to explore the capacity of equine breed types to perceive, respond, and interact with visual and auditory cues given by humans. Our hypotheses were tested using object-choice tests, where an animal must use a cue to make a correct choice to earn a reward. The primary hypothesis was that domestic equids could learn a task by using positively reinforced visual and auditory human-given cues. We hypothesized that combined cues would improve the likelihood of correct choice (approaching the bucket with the reward), but that horses' responses would be random when presented with conflicting cues. Previous object-choice tests have suggested that environmental and genetic factors are influential [3]. Given this, we predicted that ponies would outperform horses, as associations of learning ability with equine breed type and work history suggest ponies, renowned to be comparatively intelligent and adept at learning, with a history of selection for working in human-horse relationships involving traits such as low anxiousness and excitability/emotionality, and high obedience and patience, should be better able to use both visual and auditory cues than horses that are bred and trained predominantly for physical characteristics [9–12].

2. Materials and Methods

2.1. Subjects

Twenty subjects (10 geldings, 10 mares, 5–20 year old), 10 of each equine breed type (horses vs. ponies, equally distributed between sexes) participated. Subjects were from the Claremont Therapeutic Riding School, Western Australia, where they are involved in various activities, ridden by children, experienced riders, and disabled persons of varying experience. They have *ad lib* access to water and are fed hay twice daily. All the ponies and horses were familiar with each other because they were housed together in a large outdoor sandy paddock most of the time.

2.2. Experimental Design

A Latin-square experimental design was used for the object-choice tests investigating horses and ponies' capacity to use visual and auditory cues, presented alone and combined. Each subject was tested with each cue type (visual, auditory, and combined) over 10 tests. Then, two independent tests involving 10 trials per subject were conducted: a conflicting cue test involving subjects who performed above chance in the previous experimental tests, then a control test for side bias (all subjects). In all trials, order of cue type and cue presentation side was randomized (with the constraint neither two sides nor cue types were presented more than two times consecutively). For each trial, latency to make a choice was recorded.

Tests were conducted over 4 weeks in June. Ten subjects were tested per day, with the 2 days interval between tests for a given test phase (habituation, single and combined cues, conflicting cues, control trials), with each test phase conducted in successive weeks. Tests were conducted on

site in a familiar small outdoor paddock (20 × 30 m), located adjacent to the riding arena and main paddock. Subjects were within sight, sound, and scent of their herd companions, preventing separation anxiety associated with social isolation. Each horse was habituated with the test procedure over four trials (see Section 2.3). For all tests, the handler was a familiar female preventing horses from being stressed from handling by an unfamiliar person. The experimenter was relatively unfamiliar, ensuring responses validly reflected horses' abilities to respond to the experimental treatment that is the cues, in isolation from the effect of subject's differential experience history of the cue-giver. Between testing days, carrots were placed in the buckets to saturate the buckets with carrot odor, preventing subjects using olfactory cues during experiments.

2.3. Habituation to the Test Situation and Training for the Object-Choice Tests

Before the experimental tests, all subjects were habituated to the test situation, apparatus, and experimenter. The procedure was similar to the testing procedure (below), except before release at the starting point, and ensuring she had the subject's attention, the experimenter gently waved the carrot and dropped the carrot audibly into a bucket. The subject was then given a maximum of 5 minutes to reach the baited bucket and consume the reward. Trials were repeated four times, with the carrot placed on left and right sides equally in randomized order. These trials enabled subjects to learn a food reward could be obtained from approaching one of the two buckets. If subjects exhibited fear, anxiety, or were unable to grasp the task (did not approach the bucket to obtain the reward), they would have been deemed unsuitable to participate in experimental phases. Performance of all initial 20 subjects during these trials indicated that they were suitable for testing.

2.4. Procedure of Object-Choice Tests

Object-choice tests—the standard tests for assessing animal's ability to understand human-given cues [3]—required subjects to use cues provided by the experimenter that signaled the location of a food reward (positive reinforcer) hidden in one of two buckets. At the start of each trial, the handler led the animal by the halter to the starting point 4 m away from the experimenter (Fig. 1), positioned behind a fence to avoid experimenter-horse contact. Two white 9 L buckets were placed 1 m to the left and right of the experimenter inside the fence. Sides of buckets were high enough to prevent subjects seeing the bucket's contents. One of the buckets was baited with a piece of carrot, whereas the subject was led to the starting point and facing the opposite direction. Once the subject was face-on and attentive toward the experimenter, the handler unclipped the lead-ropes, turned 180° and maintained a passive posture for the duration of the trial; consequently, the handler was unaware which bucket was cued and contained the carrot. This procedure prevented confounding the subject's response due to the handler unconsciously cuing horses—which are renowned for their ability to pick-up on subtle body cues

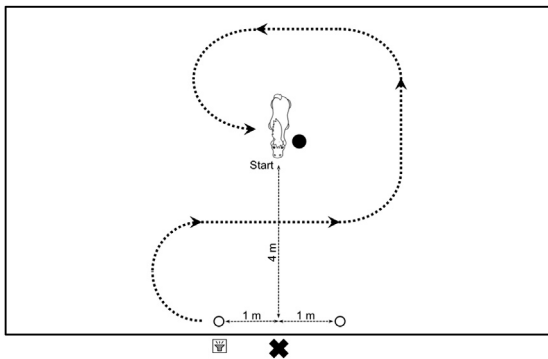


Fig. 1. Object-choice tests set-up and procedure. For example, to test a single cue, the handler (black circle) released the horse, whereas the experimenter (black cross) either pointed at one of the two buckets (open circles) containing a carrot or a sound was produced with a speaker (speaker symbol) located behind the bucket containing the carrot. At the end of the test, the handler walked the horse back to the start following a figure-of-eight (dashed line). The same handler performed all the tests.

[13]. After the subject's release, the experimenter immediately gave the cue(s). Subjects were allowed 2 minutes maximum to make a choice. Subjects choosing the cued bucket were rewarded with the piece of carrot and praised verbally and stroked by the handler on collection. Subjects choosing the wrong bucket were not rewarded and were prevented from going to the other baited bucket. Latency—defined as the time between release and choice—was recorded with a stop watch. At the end of each trial, the handler collected the horse and walked it back to the starting point in a figure-of-eight, which reduces side bias [14]. Modifications of the procedure specific for each test are described below.

2.4.1. Cues

The visual cue involved the experimenter making small up-down motions with one arm while pointing at the baited bucket. The auditory cue consisted in a recorded double clicking of the tongue played 3 times with 3-second intervals at 78 Db at 1 m using an iPod (Apple, Cupertino, CA, USA) connected to a sound speaker hidden in a box behind the reward bucket, whereas the experimenter adopted a passive stance, eyes directed ahead, providing no visual cues.

2.4.2. Object-Choice Tests with Visual and Auditory Cues, Presented Alone and Combined

All subjects were tested with each visual, auditory and combined cue type 10 times per cue type, in semi-randomized order and side over two consecutive days (15 trials per day per subject).

2.4.3. Conflicting Cue-Type Tests

Conflicting cue tests, 10 per subject, investigated how subject's respond when auditory and visual signals provide conflicting information: if subjects preferentially used a particular modality and/or preferred a particular side. Both the visual and auditory cue were presented concurrently, however, were directed at opposite buckets. In half the

trials, the baited bucket was associated with the visual cue, whereas in the other half of the trials, the auditory cue corresponded with the reward. Cue and side associated with the reward were randomized. Ten subjects (two male horses, two female horses, three male ponies, three female ponies) proficient at using the visual and auditory cues to locate the hidden food reward participated in conflicting cue tests. The criteria for proficiency was defined as a subject choosing correctly in ≥ 18 out of the 30 trials per subject (proportion differing from random choice at $P = .08$).

2.4.4. Tests for Side Biases

After conducting the experimental trials, all subjects participated in 10 trials testing for side biases. The procedure was that of the object-choice tests except no cue was given, and both buckets contained a carrot. The experimenter adopted a passive stance looking ahead.

2.5. Data Analysis and Statistics

Statistical analyses were performed in R, version 3.1.0. Statistical significance was defined as $P = .05$ and a tendency toward significance as $P = .05-.1$. Proportional data were arcsine transformed (Shapiro–Wilk test, $P < .05$). Mean proportion of correct choices between testing days was compared using a 2×2 Fisher exact test. No effect of day (learning) was found; hence, blocking for test day was not required.

For all tests, effects of explanatory factors (cue type, gender, breed type) and potential interactions on proportion of correct choices were analyzed using binomial generalized linear mixed models. Identity was included as a random term. Significant effects and interactions were retained in the final model. Proportion of correct choices for each cue type and across all three cue types was tested against chance (50%) with one-sample t tests. Difference in proportion of correct choices according to side for each cue type was tested with two-sample t tests (two tailed).

At the individual level, an animal was deemed to perform above chance ($P < .05$) if they made 19 or more correct choices out of the total 30 experimental tests and deemed to exhibit a side bias or cue-type bias if they made eight or more choices out of the 10 trials in the lateralization and conflicting cue tests, indicated by simulating binomial probabilities.

Effect of breed type, gender, cue type and outcome (correct/incorrect) on latency, and difference between factor levels was analyzed using linear mixed-effects models. Identity and trial number were random terms, and they were included to minimize possible effects of pseudo-replication. ANOVAs determined significance between levels of factors with significant effects [15]. Latency in experimental versus conflicting tests was compared with a two-tailed paired t test.

2.6. Ethics Statement

This study was approved by the UWA Animal Ethics Committee (Permit no. RA/3/100/1319) and complied with Australia's relevant legislation on use of animals in research

and guidelines for humane and ethical animal treatment. Informed consent for using the horses and ponies in the study was given by the Board of Claremont Therapeutic Riding School.

3. Results

3.1. Object-Choice Tests with Visual and Auditory Cues, Presented Alone and Combined

The correct bucket was chosen above chance level for each cue type (Table 1). Proportion of correct choices overall (0.62 ± 0.020) was also above chance level ($t_{19} = 4.0874$, $P = .0003$). There was no effect or interactions between cue type, breed type, and gender on proportion of correct choices ($P > .05$). There was an effect of breed type for the visual cue ($\chi^2_1 = 5.279$, $P = .022$), with ponies outperforming horses ($P = .044$; Fig. 2). Subjects did not exhibit a side bias ($P > .05$).

Total correct choices per subject ranged from 7/30 to 22/30. Seven subjects (35%) achieved a score for correct choices significantly greater than or equal to chance level.

3.2. Conflicting Cue-Type Tests

Ten subjects passed the criteria for participating in the conflicting cue-type experiments. Overall subjects did not preferentially choose a particular cue-type or side (Table 2), although three subjects displayed a cue-type preference for the visual cue, including a horse ("F") also displaying a left side bias.

3.3. Tests for Side Biases

With no cue presented, side chosen was random (mean preference for the right bucket 0.45 ± 0.056). However, geldings exhibited more left-side choices than mares (0.66 ± 0.079 vs. 0.44 ± 0.065 ; $P = .028$). Only one horse exhibited a significant side-bias, consistently choosing the left bucket regardless of whether a cue was present. Despite four subjects exhibiting side biases in the absence of cues, they did not have a side bias when cues were present in experimental trials.

3.4. Latency to Make a Choice

There was an outcome \times breed-type interaction on latency to make a choice: ponies had faster response times than horses when making incorrect choices ($F_{1,560} = 8.75$, $P = .003$; Fig. 3). There was a main effect of

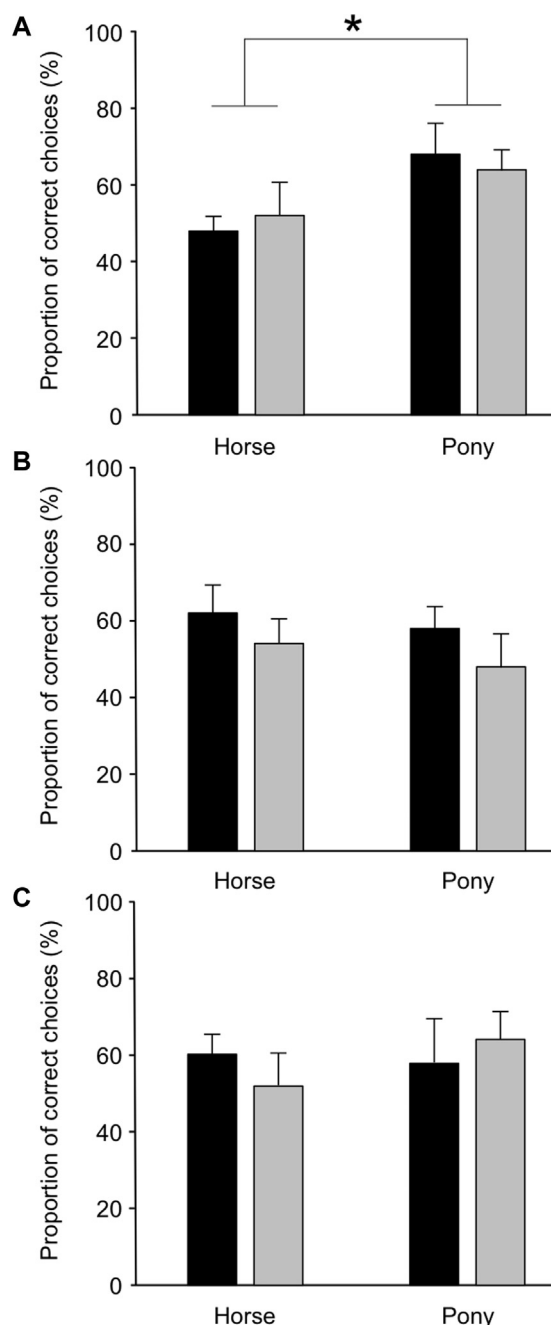


Fig. 2. Average proportion of correct choices during object-choice tests by geldings (black bar) and mares (gray bar) for horse and pony breed types responding to (A) the visual, (B) auditory and, (C) combined cue types. * denotes a statistically significant difference ($P < .05$). Error bars represent standard errors. $n = 5$ for each equine breed-type \times gender.

Table 1

Average proportion of correct choices responding to visual, auditory, and combined cue types in object-choice tests (transformed data, mean \pm s.e.). P values were calculated using one-sample t tests on arcsine-transformed data. $n = 20$ subjects. Degrees of freedom per cue type = 19.

Cue Type	Average Proportion Correct \pm s.e.	t	P
Visual	0.63 ± 0.047	2.875	.004
Auditory	0.61 ± 0.045	2.406	.013
Combined	0.64 ± 0.054	2.715	.007

outcome on latency: subjects were faster at making a choice when correct (14.4 ± 0.831 seconds) than incorrect (33.6 ± 1.94 seconds; $F_{1,560} = 6.54$, $P = .011$; Fig. 3). There was neither main effect nor interactions for gender and cue type on latency. Latency did not differ between conflicting cue tests and the visual, auditory, and combined cue tests ($t_9 = -0.591$, $P = .569$).

Table 2

Preference for a cue type or side by subjects for conflicting cue-type tests.

ID	Breed Type	Gender	Proportion of Visual Versus Auditory Choices ("n" of 10) ^a		Proportion of Left Versus Right Choices ("n" of 10) ^a	
			Visual	Auditory	Left	Right
A	Horse	Gelding	0.3	0.7	0.4	0.6
B	Horse	Gelding	0.5	0.5	0.3	0.7
C	Pony	Gelding	0.7	0.3	0.4	0.6
D	Pony	Gelding	0.7	0.3	0.5	0.5
E	Pony	Gelding	0.8 ^b	0.2	0.3	0.7
F	Horse	Mare	0.89 ^b	0.11	0.89 ^b	0.11
G	Horse	Mare	0.5	0.5	0.5	0.5
H	Pony	Mare	0.3	0.7	0.4	0.6
I	Pony	Mare	0.8 ^b	0.2	0.3	0.7
K	Pony	Mare	0.3	0.7	0.4	0.6
Mean ± s.e.			0.6 ± 0.07	0.4 ± 0.07	0.4 ± 0.06	0.6 ± 0.06

^a "F" did not make a choice in one of the trials, so for this subject, n = 9.

^b A significant bias toward a particular cue type or side.

4. Discussion

Our study confirms that *Equus caballus* are able to use visual and auditory cues in object-choice tests. These data indicate that horses and ponies respond equally well to vision and sound cue to locate food when presented alone and not better when visual and auditory cues are presented together. Our results suggest genetics may influence performance because ponies were faster and achieved higher scores especially in responding to visual cues, compared with horses. Despite auditory stimuli being important to horse perception, most studies have focused on visual stimuli [1]. Our study provides the first demonstration that horse and ponies can use auditory cues as successfully as visual cues. Moreover, the auditory cue we tested is relevant for practical applications: it is easily reproducible and similar to cues used in the popular clicker-training method [16].

Surprisingly, when visual and auditory cues were combined to signal the reward bucket, neither success nor

latency to make a choice was improved compared when using either cue when presented alone. Unexpectedly, response times were not delayed when visual and auditory cues were presented concurrently but gave conflicting directions. Our results of similar rates of successfully using visual or auditory cues, no latency differences between cue types, and no bias in cue-type when subjects failed to make a choice, indicate that overall there was no bias toward a particular cues (visual/auditory). This suggests training horses for a task can involve either visual or auditory cues. Additionally, because the combined cue did not enhance success in the object-choice task, this suggests combining cues when training horses is redundant.

As hypothesized, overall subjects chose randomly when different cues presented conflicting information, possibly because having learned through the training period, a reward can be obtained from one of the buckets regardless. Nevertheless, three of the 10 subjects in conflicting-cue tests, their choice was biased to responding to the visual cue (expected from dominance of visual spatial information in horses [17]). A potential explanation for this observation is that when different cues are presented concurrently, horses may selectively focus on one cue type. Therefore, it is possible that when a cue is given in a situation with background stimuli (noises, gestures by surrounding humans), horses might respond to the extraneous environmental stimuli rather than to the cue intentionally given by a human.

Equine learning theory suggested conflicting signals should increase latency to respond by producing a "blocking" effect and creating uncertainty when horses are confronted with different cues giving conflicting information [18]. In our study, latency to make a choice did not increase when subjects were presented with conflicting cues compared to when presented with nonconflicting cues (alone or combined). This might be the consequence of our subjects, having learned the association between a cue and the food reward, was adequately motivated to make a choice. Another possibility is that despite interactions between processing visual and auditory stimuli in the brain, horses selectively attended to one cue type, enabled by some separation of spatial processing of visual from auditory information, thereby preventing a "blocking" effect [17].

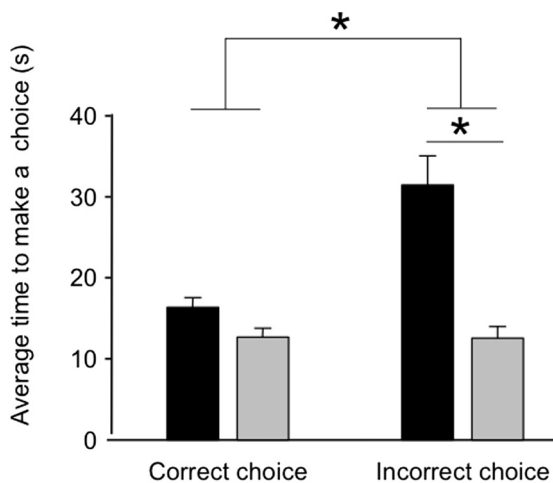


Fig. 3. Effect of subject's choice outcome (correct or incorrect) on latency to choose (time, in seconds), over the single visual, single auditory, and combined cue-type object-choice tests (mean ± s.e.) for ponies (black bar) and horses (gray bar). * denotes a statistically significant difference ($P < .05$).

Despite breed-type differences in perception, reactivity, concentration, emotivity, fearfulness, motivation, and learning ability, breed type is rarely systematically addressed as a factor in cognitive tests [19]. In our study, ponies had higher success responding to visual cues, and shorter response times overall, especially during incorrect choices. Additionally, overall, more ponies passed the criteria for demonstrating reliable competency at using cues (60% of subjects), a pony achieved the highest score, and almost all instances across cues when a subject failed to make a choice were by horses. These breed-type differences may result from the fact that ponies have higher general motivation levels to solve the task and/or were more attracted to the food reward [20]; in fact Thoroughbreds—the predominant equine breed type in our study—are known to exhibit low food motivation [21]. Additionally, ponies being generally bolder than horse breeds like Thoroughbreds can also explain ponies' lower latencies [12,22]. In addition, exposure to management conditions (e.g., box housing) likely to have been experienced by the horses because they were ex-racehorses [23] could have reduced cognitive abilities of the horses [10].

Subjects did not display a side bias (with the exception of one mare); moreover, subjects lateralized when cues were absent had a random side preference when a cue was available. Hence, subjects comprehended responding to the visual/auditory signals resulted in a reward, despite horse's innate tendency to attend primarily to spatial cues and previous reports of high incidences of laterality [8]. Consistent with reports of left-biases in males [24], in the absence of cues, geldings exhibited higher left-sidedness than mares.

Our results confirmed horses can use visual cues in object-choice tests, as previously suggested [4–7]. However, success rates were lower in our study, likely because methodology, experimental design, and testing environment in previous studies biased results toward higher success rates. Contrasting with previous studies, in our study, only one familiar handler was used, and the handler was not aware of the baited buckets location and faced away from the horse and experimenter during the tests so to avoid the “Clever Hans Effect” [13]. Additionally, different testing conditions complicate direct comparisons between object-choice studies. Our subjects were trained to the object-choice setting a few days before tests commenced, whereas in Maros et al. [4] after the “pre-training phase,” each subject had two additional “warm-up” trials before a test, involving seeing the baiting of a bucket. Additionally, Maros et al. [4] repeated the cues if subjects approached the experimenter, wandered, or failed to leave the starting point after 3 seconds, and subjects could have observed baiting of the bucket. Similarly, the rate of success could have been overestimated in Proops et al.'s studies [6,7] because subjects had only one score per cue-type but were given three opportunities to respond, and subject's failing to make a choice were excluded from analyses. In our study, repeating trials 10 times per cue-type in randomized order for each subject provides high confidence in our results and makes the criteria for success stringent. Higher success rates would be expected if each cue type was presented successively owing to greater consistency and predictability reinforcing it [25].

Previous object-choice studies proposed horses can only use cues providing local stimulus enhancement at the time of choice and are limited to using simple spatial orienting mechanisms to respond to cues [4,6,7]. Our results challenge these notions because in our study, subjects were successful at using cues where the pointing cue was away from the target (50 cm), the auditory cue was short (a few seconds), and side was randomized. This suggests processes involved in the horse's performance in object-choice tasks likely involve more cognitive comprehension than simple spatial orientation, involving decoding the cue's referential nature [3]. Because choice certainty closely correlates with both decision accuracy and reaction time, faster responses during correct choices may indicate subjects were more confident, suggesting subject's demonstrated awareness and cognitive sophistication in apprehending the task [26].

5. Conclusions

Horses and ponies were overall equally adept, motivated, and/or responsive to auditory and visual signals; however, ponies were more successful than horses at using visual cues. Combining cues involving different sensory modalities did improve neither success rates nor latency to make a choice. Confronted with auditory and visual cues presenting conflicting information, most subjects had no preference for a particular sensory cue and rather chose at random, however, some individuals tended to preferentially respond to the visual cue. No difference between successfully using visual and auditory human-given cues suggests when training or interacting with horses using either cue type should be equally effective. However, people should be alert to sounds and movements of their bodies because domestic equids are responsive to both. The animals' proficiency at using auditory cues means these can be used providing visual cues is difficult for example for disabled riders. Our study suggests that ponies and horses have a similar capacity to be responsive to informative signals involving either hearing or vision and make decisions based on potentially referential or informative human-given cues.

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Contributors: A.(K.)P. has contributed to all aspects of the study including the conception of the study, the experimental design, the animal experimentations, the data analysis, and the production of the manuscript. C.N. has contributed to the conception of the study, the design of the experiments, the data analysis, and the revision of the manuscript. D.B. has contributed to all aspects of the

study including the conception of the study, the experimental design, the animal experimentations, the data analysis, and the production of the manuscript.

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