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Judgement bias, cognitive skills and personality in the miniature donkey (*Equus asinus*)

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Sammanfattning
Abstract

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Nyckelord
Judgement bias, Cognition, Memory, Spatial Learning, Personality, Donkey

Table of Contents

1. Abstract

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Key words

Judgement bias, Cognition, Memory, Spatial Learning, Personality, Donkey

2. Introduction

Motivated by past experiences, current life conditions, personality or state of mind, we create expectations and accommodate our actions. Humans develop judgement patterns for particular situations, sometimes leading to distorted perception and illogical interpretation of events (Haselton et al., 2005) – cognitive bias that may involve attention, memory, judgment and risk assessment for example. In non-human animals, the term judgement bias is commonly used to describe how an individual reacts to an ambiguous stimulus compared to a determined positive or negative stimulus (Gygax, 2014; Mendl et al., 2009). Cognitive bias have also been defined as all cognition affected by an emotional state and named "affect-modulated cognition" by Crump et al. (2018). Mendl et al (2009) suggested the term "affect induced cognitive bias" to describe an area of study that investigates the influence of affective state on information processing in animals. As it has been observed in human and non-human animals, the emotional state can affect cognition (Mendl et al., 2009). A state can be defined within a scale of how much an individual has to do in order to cope with the environment, and how well coping attempts succeed (Broom, 1991). Under this definition, a poor state would be that in which a lot of effort (e.g. amount of energy spent) has to come from the individual in order to cope with a situation, or a case in which the individuals' attempts to cope repeatedly fail.

Emotion is hard to define and tricky to analyze under any definition, but here I think of it as discussed by Paul & Mendl (2018): an internal state of an individuals' central nervous system, elicited by instrumental reinforcers, that gives rise to physiological, behavioral and cognitive responses. Instrumental reinforcers that can be something an animal will work for (positive) or something an animal will work to avoid (Rolls, 2014). Indeed, emotions set a mood that regulates incoming stimuli, focusing the attention on the mood-related aspects of it (Paul et al., 2005). Consequently, they are in control of what the individual regains in its memory, shaping the processing of information and the accuracy of the conclusions (Blaney, 1986; Bower & Cohen, 1982; Wright, 1982). Mood has been defined as a relatively enduring affective state that arises when an experience in one context modifies the individual's reaction to future events (Asher et al., 2016).

Additionally, it is argued that some of these effects may be the result of trait-related conditions, as well as or instead of, state differences in affect, in human cases (Mineka et al., 1998; Mogg and Bradley, 2005). A trait is here defined as a component that, in order to be favored by natural selection, must improve an animal's fitness (Griffin et al., 2015). Affect refers to an experienced emotion, which can be defined as a stimulus-directed affective state, it consists of behavioral, physiological, and cognitive components, and may occur outside awareness (Crump et al., 2018). Humans reporting negative affective states, e.g. depression or anxiety, tend to make negative judgements about the future, and are commonly referred to as “pessimistic” (Mineka and Sutton, 1992), while people reporting positive states tend to judge the same ambiguous information with an “optimistic” outcome (e.g. Weinstein, 1980; Dawkins, 1993; Macphail, 1998; Eysenck et al., 1991; Wright and Bower, 1992; MacLeod and Byrne, 1996; Nygren et al., 1996).

Early studies on cognitive bias, in non-human animals, focused on the correlation between different environments (e.g. enriched vs barren housing) and the animal’s response to ambiguous stimuli (rats: Harding et al., 2004; Burman et al., 2008; Enkel et al., 2010; starlings: Matheson et al., 2008). Harding et al. (2004), who published study regarding cognitive bias as an animal welfare indicator, concluded that rats living in unpredictable environments were more pessimistic, displaying behaviors that suggest negative bias, such as fewer and slower responses to ambiguous stimuli. Likewise, Burman et al. (2008) found that rats held in barren environments responded with less excitement to an ambiguous stimulus, when their responses were compared to those of rats being held with enriched surroundings. Analogous studies mentioned ahead had similar results, suggesting that negative affective states are associated with negative cognitive bias in non-human animals too. However, in those cases, behaviors are harder to interpret, there is a lack of positive affective state measures and many of the criterion lack a priori hypotheses for how they should change according to the individual’s emotional state (Mendl et al., 2009). Nonetheless, cognitive bias tests have been set as a very reliable and non-evasive indicator of animal welfare, for several mammal (e.g. Bethell et al., 2012; Verbeek et al., 2014), avian (e.g. Matheson et al., 2008; Wichman et al., 2012; Lalot et al., 2017) and invertebrate species (e.g. Bateson et al., 2011).

Studies have found that negative affective states are not only associated with shifts in judgement but also other cognitive processes such as attention and memory, both in human and non-human animals. The understanding from human studies is that cognitive function is likely enhanced by short-term exposure to low levels of corticosterone, and impaired by short-term or sustained exposure to higher levels of this stress hormone (Lindau et al., 2016). Receptors for the latter, glucocorticoid receptors, are abundant in two important brain regions for the modulation of learning and memory - the hippocampus and the amygdala (Lindau et al., 2016). Mendl et al. (2009) summarized the various reports asserting that people in a negative affective state (e.g.

anxiety) show enhanced attention to threatening stimuli, when compared to people in a more positive state. Crump et al. (2018), confirmed this evidence and reviewed the inconclusive results for animal species, stating that attention biases are observed, related to welfare and very informative across animal taxa. There are numerous indications that affective state influences memory retrieval, with happier people being more likely to recall positive memories and unhappy or depressed people more likely to recall negative ones (Mendl, 2009; Paul et al., 2005). This question was analyzed by Paul et al. (2005), who discussed the indisputable role of the amygdala in storing emotional memories. Elevations of the hormones catecholamines and/or glucocorticoids in the hippocampus are associated with enhanced memory and learning throughout any kind of event: positive, negative or neutral (Hamann et al., 1999; Lindau et al., 2016). However, if those increases are too big, they will have disruptive effects on memory, which makes it hard to interpret results (Paul et al., 2005; Lindau et al., 2016).

Furthermore, it has been noted that the term cognitive bias suggests an irrational decision-making process, and although it is often associated with detrimental emotional states, a biased decision may prove itself adaptive if taken into consideration with the information on which the decision is based (Mendl et al, 2009). There is a possibility that emotions have developed to meet different environmental demands, leading to links with different cognitive methods (Oatley & Johnson-Laird, 1987). Anxiety facilitates vigilance and preparation for action (Mathews & Klug, 1993), while depression triggers a sharper reflection of events that have led to failure or loss (Mineka et al 1998) - both presumably adaptive qualities. Ultimately, attending to arousing stimuli, regardless of whether they are positive (e.g. sexual, food related) or negative (e.g. threat) is more likely to contribute to survival and reproduction than attending to neutral stimuli (Paul et al., 2005).

In the hope of finding more about the function of cognitive bias, emerging research is looking at its possible link with personality. Is there a personality component to cognitive bias? At what level are individuals bound to be optimistic or pessimistic, independently of their current life circumstances? Questions that are yet to be answered both in human and non-human animals, and motivated the present work. Réale et al. (2007) defined animal personality as the differences between individuals' average level of behavior that are repeatable across time and contexts, thus the same definition was adopted in this study. Personality has been associated with cognition in several occasions, setting a precedent for possible outcomes and interpretations of the results in this research. Amongst black-capped chickadees, exploration of novel environments has been proven to be correlated with learning speed and slow-exploring individuals may be generally more able to modify their behavior to adapt to different stimuli (Guillette et al., 2009; Guillette & Sturdy, 2011). Guenther et al. (2014) found strong positive relationships between all personality traits and learning speed in cavies, whereas flexibility was negatively associated with aggressiveness. Researchers working with zebrafish proposed that personality-related cognitive traits partake in mediating the trade-off between current and future reproduction (Brust et al., 2013). Experiments undertaken with mynas show that individuals that solved a foraging task were quicker in a discrimination learning challenge, but slower to shift their behavior when the cues changed (Griffin et al., 2013). In mice, imposed social subordination impairs exploratory behavior and general cognitive abilities including learning (Colas-Zelin et al., 2012). Nonetheless, within the same group, naturally subordinate individuals did not perform below average in learning tasks (Colas-Zelin et al., 2012). In 2016, Asher et al. found a positive correlation between proactive personality traits and optimism in pigs. Later, a similar link was found in dogs (Barnard et al., 2018).

Such questions were only recently investigated in equids (e.g. Osthaus et al., 2013; Gabor & Gerken, 2012; Hanggi, 2005) and very briefly in donkeys (McGuire et al., 2018). Horses are able of complex cognitive processes such as relational concept learning (Gabor & Gerken, 2012), discrimination, categorization, quantification and long-term memory (reviewed in Hausberger et al., 2019). They have also shown variable judgement bias, for example, when housed in either restricted or naturalistic situations (Henry et al., 2017) and when housed either in stalls or herds (Löckener et al., 2016). Additionally, fearfulness and stress in this species have been associated to shorter working memory (Valenchon et al., 2013).

Although from different lineages and having different ecologies, donkeys and horses belong to the same genus and share many similarities (Vilá et al., 2006), probably including cognitive strategies. Donkeys have been able to solve a detour task (Osthaus et al., 2013), shown discrimination learning abilities [Giebel, 1958 (cited in Waring, 2003); Proops et al., 2009] and were able to recall the location of a hidden object after a delay (Baragli et al., 2011). The only reported results for judgment bias tasks in donkeys come from McGuire et al. (2018), who looked at the performance of horses and donkeys, to conclude that rescued individuals were more optimistic than non-rescued individuals.

Miniature donkeys have been evaluated differently than other donkey breeds in physiological tests but have not been reported to be different in a way that could influence the results for the kind of behavioral tasks performed here (e.g. Samini, 2019; Hibbs et al., 2019; Altegany et al., 2017; Matthews and Taylor, 2002). However, there is a strong possibility that selective breeding has led to temperamental and cognitive differences, as manifested in horses (reviewed in Hausberger et al., 2019).

Whereas most cognitive bias studies compare results for different treatments (e.g. different housing quality, different levels of arousal, training style), here only individual differences were analyzed. Since all study subjects were under the same conditions during and at least for three years preceding the study period, any differences in their response to the trials should be related to early life experiences, personality, or short-term stress. Thus, it was hypothesized that individuals should vary in their judgement bias upon an ambiguous stimulus, and that those bias would be associated both to personality factors and other cognitive processes. Particularly, nervous individuals were expected to be more pessimistic and consequently to perform worse in the cognitive tasks. The capacity to, based on aspects of personality, predict that an individual might more often than others experience negative emotions, and for that reason have impaired cognitive abilities, is of extreme importance for animal welfare studies and care takers who interact with animals on a daily basis. For the same reasons, it is relevant to understand at what level individuals living under good conditions and showing low stress levels might experience negative mental states and low expectations towards new stimuli.

3. Materials and Methods

3.1. Study site

The experiments were carried out at the T.S. Glide Ranch in Davis, California, USA - a charitable foundation to protect animals, wildlife, agriculture and the environment of Yolo County. The ranch comprises several housing facilities and barns, as well as pasture and agricultural fields. An outdoors test arena was implemented in the area, so that it was as isolated as possible, but not isolated enough to hinder the animal's displacement. Being in the vicinity of a working farm, the arena was not detached from potential environmental stimuli for the animals. However, auditory and visual contact with conspecifics seemed to reduce the individuals' habituation period and stress levels resulting from their inevitable separation from the social group during the behavioral tests.

California is characterized by a Mediterranean climate where precipitation occurs mostly in the winter and cold temperatures are rare. Vegetation in Mediterranean-type climates is composed by sparse woodlands, grasses and shrubs (Seager et al., 2019). The climate in Davis follows the latter description and the region is defined by flat lands that have frequently been converted into agricultural use.

The study was conducted between September and February, therefore covering two very different three months periods – long, warm, and dry days from September to November, and shorter, cold and often rainy days from December to the end of February.

3.2. Animals

The study subjects (n=8 females, 4 males), ranging in age from one to twelve years (Appendix 1, Table 1) were part of a group of eighteen rescued miniature donkeys, currently being kept as pets in shared pastures, with other donkeys, goats and cows. The animals' daily activities included almost no human interaction besides feeding, which happened twice a day (early morning and evening), occasional visits by the veterinarians and monthly blood collection for research purposes. The donkeys had not undergone any prior systematic training previous to this study. The group was rescued from two different locations, both private owners that could no longer care for them, moved to a temporary location, and finally brought to the Glide. Some individuals were rescued as adults, others as foals, and two were later born at the ranch (Appendix 1, Table 1). At the time of the study four individuals were separated from the group, in a smaller barn, to facilitate social dynamics.

Six donkeys did not reach the training level required for participating in the tests, within the first three weeks, and therefore are not represented in the data. The remaining twelve individuals went through approximately three weeks of training, or long enough to show increased cooperation with the researchers and reduced fear levels associated with the test arena and material. During this period, each individual was brought to the test arena for approximately half an hour per day, led at a walk with a halter and leadline, and allowed to interact, investigate and become familiar with the test apparatus.

3.3. Data Collection

3.3.1. Weather and Training

Wind, temperature and humidity were recorded for each test. Food was used as a motivator to encourage donkeys to complete the tasks in all experiments. A food mix of apples, carrots, dry treats and grain was used in order to attend to various individual preferences. Training sessions included first time interactions and familiarization with the food bucket, which finally led to a choice of a smaller, pan-shaped test bucket (Figure. 1) that reduced fear related responses associated with the loss of peripheral vision while reaching for the food. Contrary to what has been done in previous studies, the food bucket did not have a lid during the tests. The reason for this being that it not only increased fear as it did not decrease latency to find the food or influence the individuals' choices, during the pilot studies. There were always two experimenters and one donkey present during experimental trials. Training and habituation sessions often included more than one donkey in the test arena, to promote social buffering and social enhancement towards exploration of new objects.

Figure 1. Training session with Nacho.

3.3.2. Personality

An adaptation of a previously validated questionnaire (Navas et al., 2012) (Appendix 2) was completed by ten people including three caretakers, two people that were in occasional contact to the donkeys and five experimenters that had worked with the animals for five weeks. The questionnaire was composed of a total of nineteen ordinal questions concerning specific behavioral qualities such as Concentration, Training potential, Excitability, and Vigilance, for example, which were rated from 1 to 10. To each question followed a description of the behavior, which was defined again at the two extremes of the scale.

3.3.3. Memory and Spatial cognition

To investigate short-term memory and spatial cognition, a test was adapted from a study conducted by McLean (2004). Each trial started with the individual at location A, which was walked around both sides (experimenter was always on the left of the animal) – the side with no food was always shown first – and allowed to eat from the bucket, which should have been previously hidden behind one of the walls (locations B or C) (Figure 2). The donkey was then guided back to location A and released to explore the arena. A trial was considered successful when the donkey first chose to explore the side of the arena where the food bucket was hidden (registered when the animal's head was over the D mark), unsuccessful when the donkey first chose to explore the side of the arena opposite to where the food bucket was hidden (registered when the animal's head was over the D mark) and canceled when, within the first 30 seconds, it did not cross the D mark (Figure 2). Throughout a set of ten Familiarization trials (successful or unsuccessful), locations B and C were used alternately to place the food, in a way that the same donkey should not be tested more than five times at the same location. For these trials, the donkeys were released immediately after being walked around both sides of the arena and brought back to location A (Figure 2). During this period, the animals were rested and tested later if they happened to have three canceled trials. The following group of trials (IR, Immediate release phase) was composed by four sets of ten trials, in which the individuals were released immediately after being walked around both sides of the arena and brought back to location A (Figure 2). Locations B and C were used to hide the food in a random order, guaranteeing that each donkey was tested twenty times in each one, and not more than two consecutive times in the same. All donkeys were not tested for at least twelve hours between each set. The individual's performance was evaluated for this testing phase, in order to identify which donkeys were able to memorize where the food was located. The context of the trials did not allow for the animals to be released immediately after they became aware of the food location, therefore forcing them to retain that information for thirty seconds, even during IR trials. Donkeys that succeeded (first choosing to explore the side of the arena where the food bucket was hidden) in more than 75% of the trials were qualified to move on to the next test phase (Binomial test, $p < 0,05$). Finally, six individuals went through four sets of ten trials, in which they were released thirty seconds after being walked around both sides of the arena and brought back to location A (DR, Delayed release phase) (Figure 2). Otherwise the test procedure remained the same as for the preceding trials.

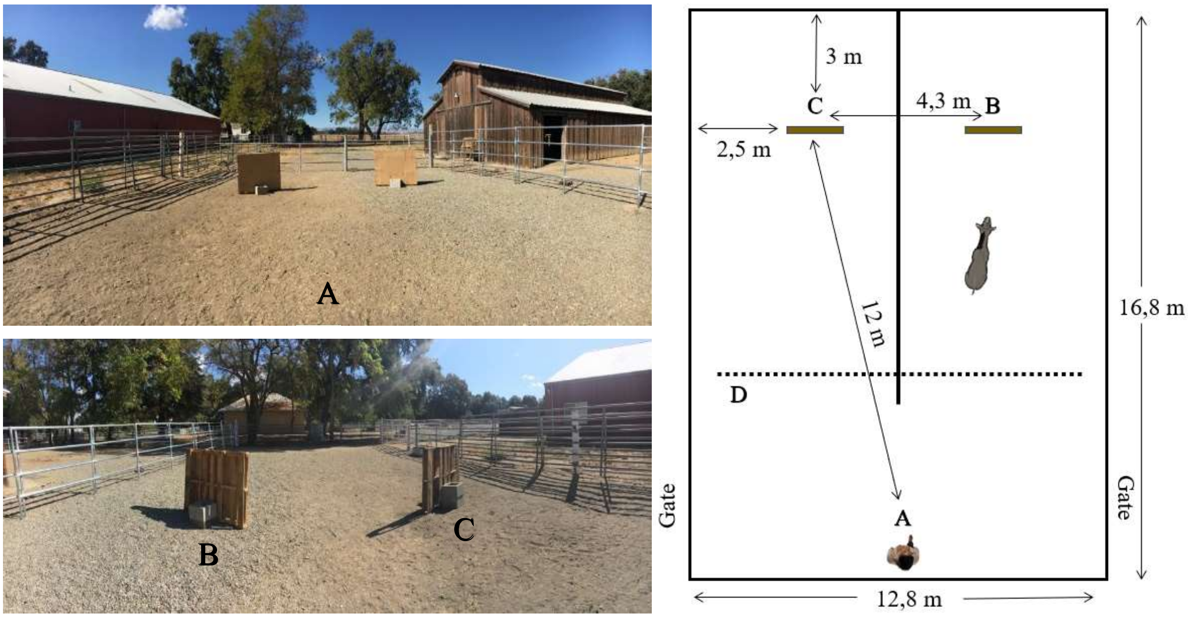

Figure 2. Experimental set-up during Memory trials. Positions of the food bucket with respective blinds (C; B), starting location (A), dividing wall, gates. A trial was considered successful if the donkey crossed the imaginary line (D) on the side of the wall corresponding to the side where the bucket was hidden.

3.3.4. Judgement bias

Following these trials, the donkeys were tested for judgement bias, following a protocol adapted from Freymond et al. (2014). During a discrimination phase the animals learned to distinguish between a positive and a negative stimulus, in this case a black bucket full of food and a white empty one. This phase included an undefined number of sets, depending on how long it took each individual to learn the distinction, each composed of five positive and five negative trials. Positive trials were characterized by having a bucket full of food on one side of the arena (sides changed between donkeys, with half the group being tested with the positive trials on the left and half on the right), while negative trials had an empty bucket on the opposite side (locations A+ or A−, Figure 3); there was always just one bucket at a time inside the test arena. The individuals were brought to point A (Figure 3) when a bucket was already positioned on location + or −. They were then released and allowed to explore and, in the case of positive trials, to feed. Latency was measured and registered as the time spent between the donkey's release at B and the moment when its throat latch was less than one meter away from the bucket. If, after three minutes, the donkey had not reached the bucket, the handler should guide it to the goal and reveal the bucket's content. Trials were considered finished after three minutes or when the individual's throat latch was less than one meter away from the goal. After each trial, while the handler brought the donkey back to the start (location A), another experimenter swapped the buckets so that the set up was correct for the following trial. Each set was composed of three positive and three negative trials, randomly ordered in a way that no more than two of the same trials followed each other (i.e. + + − − + −, + + − + − −, − + + − − +, + − − + + −, − − + + − +, − + − − + +) and the animals rested for at least twelve hours between sets. Donkeys were considered fit to move on to the treatment phase when the latency to reach the positive goal was significantly shorter than the latency to reach the negative goal (Mann-Whitney test, $p < 0,05$).

The treatment phase exposed the animals to three new buckets of different colors: dark grey, aligned to the right of the black bucket's location (+A); medium grey, aligned to an intermediate position between the black and the white bucket's location (An); light grey, aligned to the left of the white bucket's position (−A) (Figure 3). These were presented to the test subjects individually, always empty and in random order, across two sets of seven trials (+ − A+ + An – A−; − + A− − An + A+). Individual exposure to this phase was limited to two sets, in order to avoid learning (Freymond et al., 2014; Doyle et al., 2010). The remaining steps of the experimental procedure were the same as used for the discrimination learning phase.

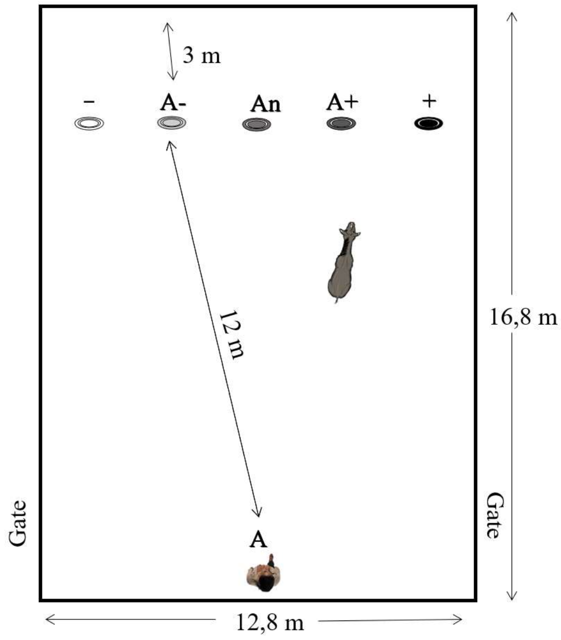

Figure 3. Experimental set-up during Judgement bias trials. Position of the positive goal (+ ; right or left depending on the donkey), the negative goal (− ; opposite side as the rewarded one), the ambiguous goals (A−, An, A+; equidistant angles between the positive and negative buckets), the start (A) and entrance gates. The latency to reach the goal was measured as the time to go from A to one meter away from the tested bucket.

3.3.5. U task

Lastly, eleven donkeys were tested with a modified U-task (Baragli et al., 2011) to further explore spatial cognition. A U-shaped barrier was designed with three different levels of asymmetry (Figure 4), two to three experimenters were present. Before the trials began, all donkeys went through a habituation phase and were allowed to explore the test arena for twenty minutes or until a successful detour to reach the food within three attempts. The test was set up during this time of habituation (feed bucket with rope, barriers 1, 2 and 3 – Figure 4). For the trials a test subject was walked in by the handler to A, a bucket with food attached to a rope was previously placed at A and, immediately after the donkey had eaten from it, the bucket was removed by a second experimenter to B. At this point the donkey could no longer reach the bucket but could still see it [as previously proven to increase difficulty to this task (Baragli et al., 2011)]. Individuals were then released and allowed to explore while the handler walked away and stood behind the imaginary line C (Figure 4). After it successfully detoured the barrier and reached the bucket, the donkey was allowed to eat and then walked back to B for the next trial. The handler stood alternately on the left or right of the animals while walking them, in a way that each animal was walked on the right for half of the trials, and on the left for the remaining ones, in order to avoid influencing the side choice during detour behavior.

This task was divided in three phases: D0 (barrier 1 – Figure 4), composed by ten trials of five minutes each or until successful detour (donkey's throat hatch less than one meter away from the bucket); D1 (barriers 1 and 2 – Figure 4), composed by ten trials of five minutes each or until successful detour, considering that barrier 2 should be placed on the right for five trials and on the left for the remaining five, following a randomly defined order that did not allow for the barrier to be on the same side for more than two consecutive trials (LRLRRLRRLL); D2 (barriers 1, 2 and 3 – Figure 4), composed by two sets of ten trials of five minutes each or until successful detour, considering that barriers 2 and 3 should be placed together, on the right for five trials and on the left for the remaining five, following randomly defined orders that did not allow for the barrier to be on the same side for more than two consecutive trials (LRRLRRLLRL, LRRLLRRLLR). Both the side chosen for the detour and the latency to reach the bucket, from the moment of release at A, were registered. Contrary to previous set ups for this task (Baragli et al., 2011), the second experimenter was not hidden behind a wall, as this was thought to increase the animals' fear levels during the pilot tests, and eye contact with the experimenter did not seem to influence their performance.

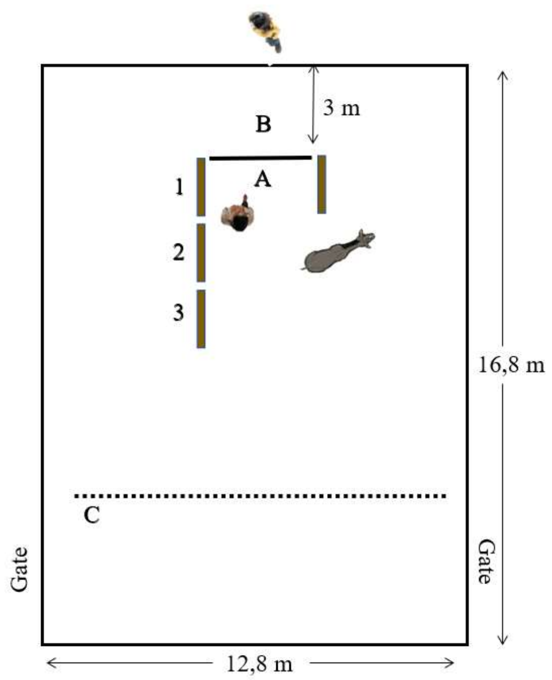

Figure 4. Experimental set-up during U-task trials. First position of the food bucket (A), which was then pulled to the second position (B), starting point (A), gates, barrier during D0 trials (1), D1 trials (2) and D2 trials (3). Latency to complete the task was measured as the time to go from A to B and trials were canceled if the donkey crossed the imaginary line (C).

3.4. Data analysis

Intraclass correlation coefficient analysis was performed to confirm intra observer reliability, between those who answered the personality questionnaire. For each individual, an average was calculated of the scores given by all the observers in each question. This data was then analyzed using Correlation analysis, in order to cluster the questions into groups that represented broader personality factors. The scores of the correlated questions were then averaged to calculate a score for each factor. Donkeys that completed IR, first choosing to explore the side of the arena where the food bucket was hidden in more than 75% of the Memory test trials, were qualified to move on to the next test phase (Binomial test, $p < 0,05$). Success rates were calculated for each Memory test (IR and DR trials), for each donkey, by dividing the number of successful trials by the total number of trials. Data from the judgement bias tests was reduced to an adjusted latency to approach the ambiguous stimulus (An), for each donkey. The latter was calculated considering the individual's latencies to approach the positive and negative locations, as follows:

$$\text{Adjusted score} = \frac{\text{Mean latency to ambiguous stimulus} - \text{Mean latency to rewarded stimulus}}{\text{Mean latency to unrewarded stimulus} - \text{Mean latency to rewarded stimulus}}$$

By expressing all scores as a comparison to the baseline latency of each donkey to approach the stimuli, it is possible to eliminate individual differences that are unrelated to judgement bias (Mendl et al., 2010).

Donkeys were considered fit to move on to the treatment phase when the latency to reach the positive goal was significantly shorter than the latency to reach the negative goal (Mann-Whitney test, $p < 0,05$). Standard mean latencies were calculated for the detours in D0, D1 and D2 trials, for each donkey. Side preferences for the same detours were investigated for each individual and at a group level with Binomial tests ($p < 0,05$). Correlation analysis was conducted with all the previously mentioned variables: D0, D1 and D2 latencies, IR and DR success rates, Personality scores (Spearman correlation, $p<0,05$). To assess the effect of weather (Humidity, Wind and Temperature) and time of day (Morning or Afternoon) in the tests' results, Spearman correlations ($p<0,05$) and Mann Whitney tests ($p<0,05$) were conducted. Further analysis was run to investigate correlations with the individual's Life History (Kruskal-Wallis, $p<0,05$) and Housing Situation (Mann Whitney, $p<0,05$).

4. Results

4.1. Personality

A high degree of intra observer reliability was confirmed for the Personality questionnaires (Anabelle: α=0,944; Gladys: α=0,909; Janette: α=0,917; Lucy: α=0,930; Nacho: α=0,910; Robin Hood: α=0,800; Sofia: α=0,891; Storm: α=0,910; Sunny: α=0,925; Taquito: α=0,904; Doris: α=0,928; Henry: α=0,923) (ICC, $p<0,05$). Data for the ten observers was then averaged by question, for each donkey. Correlation analysis on the questionnaire data revealed significant correlations between some of the questions (Appendix 1, Table 2), which were clustered into eleven groups and named accordingly to the personality factors they represent (the calculated scores for each individual can be found in Appendix 1, Table 3).

4.2. Sex, Age, History and Weather effects

The environmental factors registered during the tests - temperature, wind and humidity - did not have a significant effect in any of the tests' results (Mann-Whitney test, $p>0,05$) (Appendix 1, Table 4). The individuals' Life History (Rescued as adults, Rescued as foals or Born at the Ranch) did not prove to have a significant influence on either of the variables under study (Kruskal-Wallis test, $p>0,05$). Likewise, Housing conditions did not influence the tests results or any other individual variables (Kruskal-Wallis test, $p>0,05$), and Sex effects were not significant (Spearman's rank correlation, $p>0,05$). A negative correlation was found between Age and Vigilance ($r_s=-0,637$, $p<0,05$) (Figure 5).

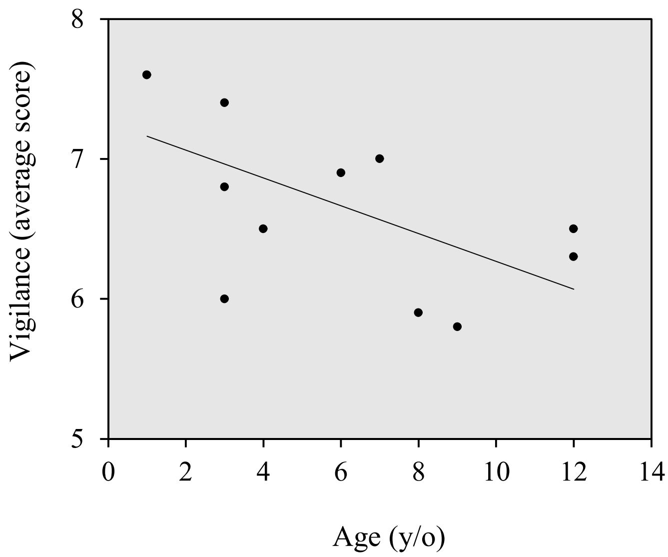

Figure 5. Scatterplot and trend line of the correlation between Vigilance and Age. Each dot represents an individual, with Vigilance scores obtained from the Personality questionnaire.

4.3. Memory and Spatial cognition

Half of the study subjects completed IR phase with at least 75% successful trials (Binomial test, $p<0,05$). These donkeys still performed above chance level (at least 60% successful trials) during DR trials (Binomial test, $p<0,05$). Average success rate between individuals was 0,66 ± 0,06 for IR Memory trials (N=12), and 0,66 ± 0,05 for DR Memory trials (N=6).

4.4. Judgment bias

During DL phase, the group average latency to approach the positive stimulus was 30,3±34,4 seconds, and 77,2±65 seconds to approach the negative stimulus. During Treatment phase, the mean latencies to approach the stimuli were 33,7±43,1 seconds (positive), 37,9±50 seconds (A+), 85±70,3 seconds (A), 91,5±71,4 seconds (A-) and 145,5±53,1 seconds (negative) (N=12). Latencies to approach the ambiguous stimulus (An) varied between individuals and were plotted in Figure 6.

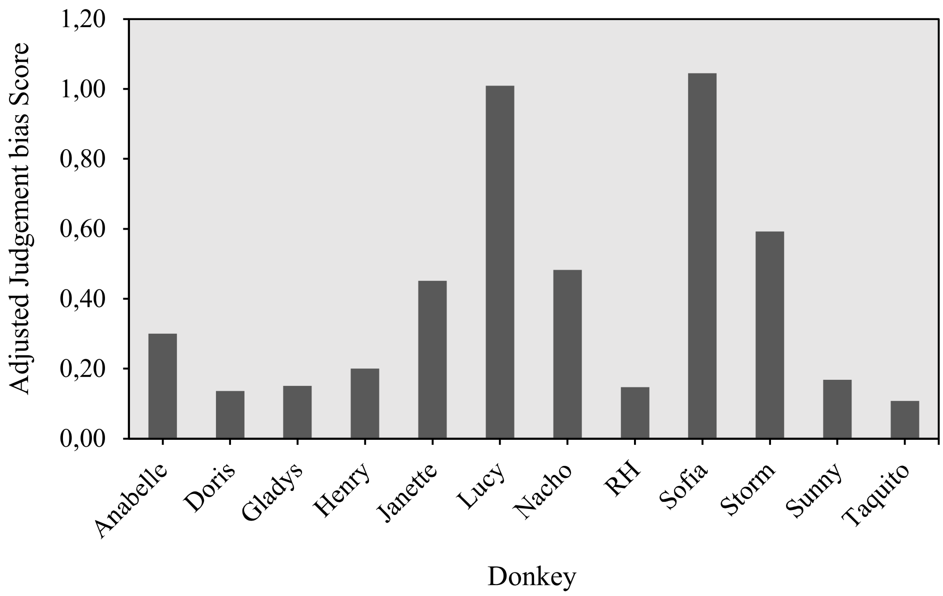

Figure 6. Adjusted Judgement bias score (calculated from the latencies to approach the ambiguous stimulus – An, during Treatment phase of the Judgement bias test) (See Methods – section 3.4.). Individuals that approach the stimulus more promptly have lower scores.

4.5. Detour behavior

The mean latencies to reach the bucket of food were 19,9s (D0 trials, N=11), 17,2s (D1 trials, N=11) and 23,3s (D2 trials, N=11). During D0 trials, nine individuals showed a side preference to go around the barrier, and the same results were observed in D1 trials (Binomial test, $p < 0{,}05$) (Figure 7). Throughout D2 trials, nine individuals showed the same side preference as they had for D0 and D1, one individual demonstrated no side preference, and one individual revealed a side preference for the shorter detour around the barrier (Binomial, $p < 0{,}05$) (Figure 8). No side preference was found at a group level (Binomial, $p < 0{,}05$).

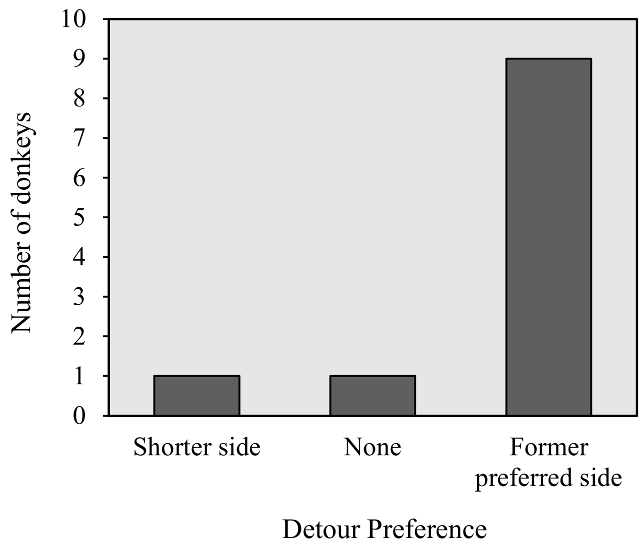

Figure 7. Side preferences during D0 and D1 trials. Three individuals showed a Right-side preference to go around the barrier, six individuals preferred to go around the Left and two individuals had no side preference (Binomial test, p <0,05).

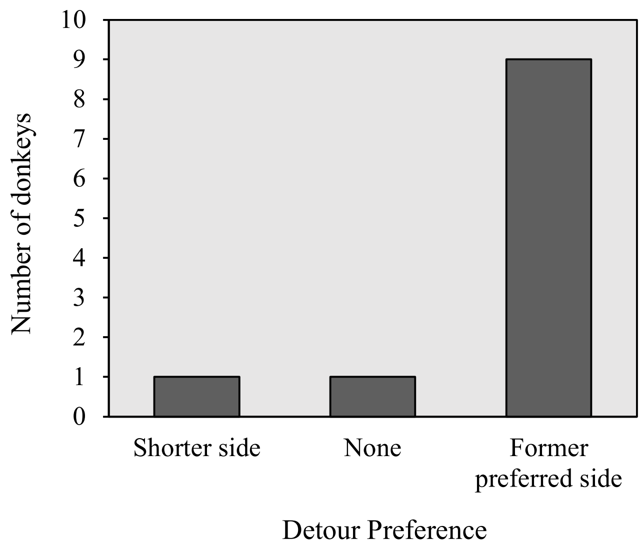

Figure 8. Existence of a side preference during D2 trials. Nine individuals kept their side preferences to go around the barrier during D0 and D1 trials, one individual showed no side preference and another one revealed a preference for the shorter way to reach the bucket of food (Binomial test, p <0,05).

4.6. Correlations

A significant correlation was found between the personality factor Concentration and the Mean Detour Latency to go around the barrier during the U-task (r_s=-0,657, p<0,05) (Figure 9), as well as between Patience and the Latency to complete the D1 phase of the U-task (r_s=0,766, p<0,01) (Figure 10). Moreover, Predictability was found to be positively correlated with Memory performance during IR trials (r_s=0,666, p<0,05) (Figure 11). Lastly, a positive correlation was found between Patience and negative Judgement bias (r_s=0,714, p<0,01) (Figure 12).

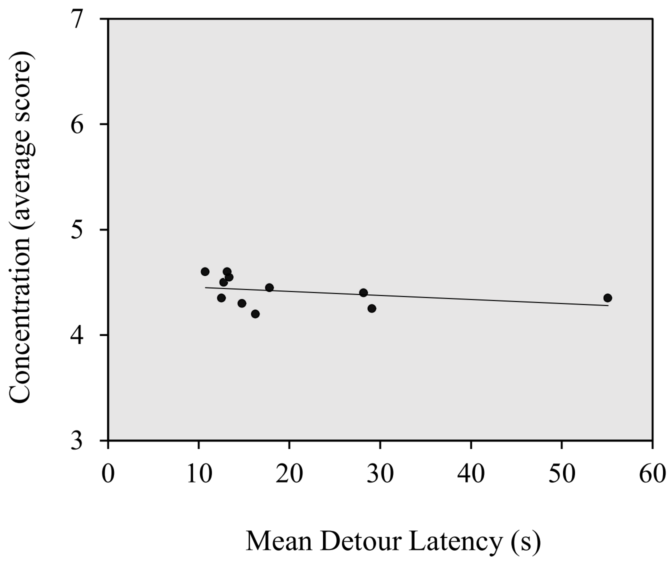

Figure 9: Scatterplot and trend line of the correlation between Concentration and the Mean Detour Latency to reach the food bucket during U-task trials. Each dot represents an individual, with Concentration scores obtained from the Personality questionnaire and the Mean Detour latency in seconds (average of the detour latencies during D0, D1 and D2 trials of the U-task).

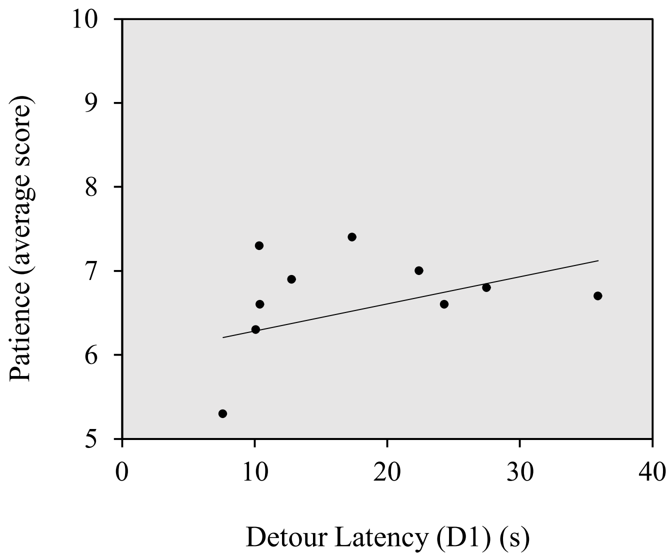

Figure 10: Scatterplot and trend line of the correlation between Patience and Detour Latency during D1 trials of the U-task. Each dot represents an individual, with Patience scores obtained from the Personality questionnaire and the Detour latency in seconds (average of the detour latencies during D1 trials of the U-task).

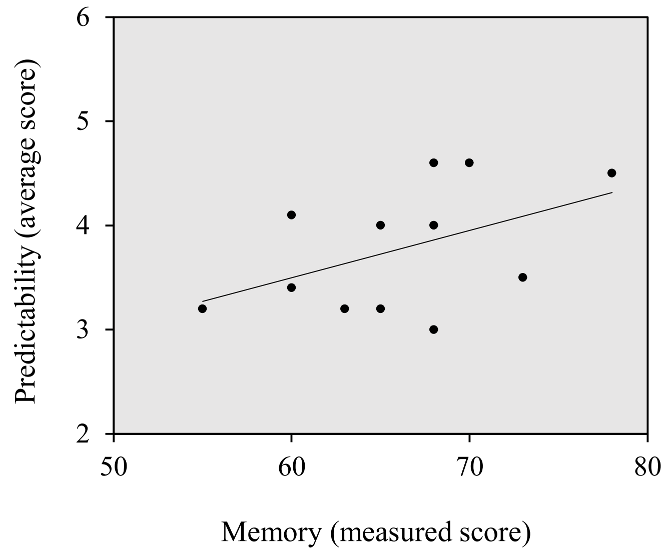

Figure 11: Scatterplot and trend line of the correlation between Predictability and Memory. Each dot represents an individual, with Predictability scores obtained from the Personality questionnaire and Memory scores during IR trials (calculated by dividing the number of successful trials by the total number of trials).

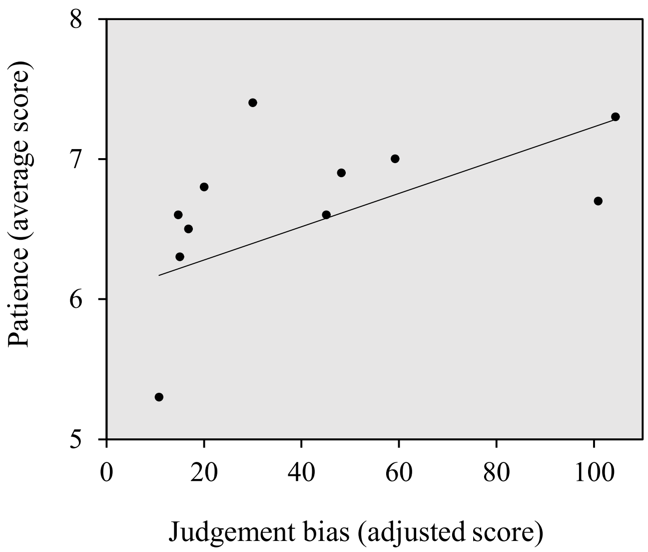

Figure 12: Scatterplot and trend line of the correlation between Patience and Judgement bias. Each dot represents an individual, with Patience scores obtained from the Personality questionnaire and the adjusted judgement bias scores (calculated from the latencies to approach the ambiguous stimulus – An, during Treatment phase of the Judgement bias test) (See Methods – section 3.4.).

5. Discussion

5.1. Personality

The analysis conducted on the questionnaire revealed correlations that might yield important knowledge about donkey personality. These are not discussed in this report as they are not directly linked with the proposed hypothesis. The factor Friendliness was calculated with the scores of questions referring to antagonistic behaviors in the context of interactions with both donkeys and humans. Nervousness on the other hand, incorporates questions that classified the individuals on their comfort with the environment in challenging situations. However, these factors are interrelated, and both refer to broader personality traits, deeming the classification ambivalent.

5.2. Sex, Age, Weather, Life History and Housing effects

All the experimental procedures were performed in an outside arena, exposed to various weather conditions, sounds, smells and visual stimulus, as well as the known presence of conspecifics. It is predicted that the results reported here would be different if tests were conducted in an isolated and more neutral environment, especially if we take into consideration that donkeys might be more sensitive to weather changes than other equids (Osthaus et al., 2018). Younger individuals possibly have different time budgets and display differing behaviors (in this case observed with Vigilance scores), when compared to adults - which was not controlled for in this study. Additional research is necessary to further develop these questions, as equid literature is very scarce on the topic.

5.3.Memory and Spatial cognition

An earlier study with donkeys (Baragli et al., 2011) revealed that their short-term memory extended for thirty seconds. The memory tests reported here indicate that miniature donkeys are capable of retaining information about the location of hidden objects, within a fairly complex experimental set, for at least one minute. In fact, the actual extent of their memory ability is likely to be much bigger (Hanggi and Ingersoll, 2009), and the reason why only half of the individuals successfully finished the task might be related to environmental distractions or problems with the experimental set up. These results are in concordance with findings about short-term memory abilities of horses (e.g. Hangi, 2010; Baragli et al., 2011).

The correlation found between Predictability and Memory may suggest that when individuals are able to better recall events, they are also more predictable. As most unpredictable behaviors, in this context, were reported to be related to fear responses, low scores for Predictability were most likely attributed to insecure individuals that, despite their progress during training on one day, did not increase their confidence and comfort for the next days, in the experimental setting. If true, the latter hypothesis might find its explanation in the individual's memory capacity to register information and retrieve it later (e.g. the fact that the experimental procedure is non-threatening). Additionally, fearful horses have been found to perform worse than non-fearful horses, in a memory task, under stressful conditions (Valenchon et al., 2013) - which would explain the results in this study, if assumed that unpredictability was related to fear.

5.4. Judgement bias

As predicted (McGuire et al., 2018), donkeys were able to learn to discriminate between a positive and a negative stimulus. The removal of food in the bucket has proven to be enough to promote discrimination learning, deeming unnecessary to use negative reinforcement for this kind of tests. Although not quantified in this study, time spent at the positive location during negative and ambiguous trials, suggested that spatial location cues might play a more important role than colour discrimination. Though horses have dichromatic vision and are able to distinguish brightness levels (Geisbauer et al., 2004), additional studies would be necessary to confirm that colour discrimination was even incorporated in the study of donkeys' cognitive process to solve this task. Individuals differed from each other in their judgement bias, as exhibited in their different latencies to approach an ambiguous stimulus. Since there were no treatment differences, the variation found between individuals must be attributed to either life history, current housing situation, personality, or a combination of these.

The positive correlation found between Patience and the Judgement bias score indicates that the individuals classified as more patient were also more pessimistic. Patience however, as interpreted by the observers, might instead reflect reduced nervousness or low exploration rates, for example. There is also a possibility that this personality factor reflects that individuals are generally slow when solving new challenges, as observed during the Detour task. From a different perspective, expectations towards some stimuli are perhaps reducing the individual's motivation to interact with them, originating the passive behavior we understand as patience. The latter has been studied as an adaptive choice of renouncing immediate benefits to acquire more valuable future rewards (Rosati et al., 2007). Maybe this behavior is not only related to the expectation of a better future reward but also, in some cases, with the expectation of no reward at all. Moreover, if we assume that individuals with generally lower activity levels may have been classified by the observers as more patient, this could partially explain why they react less promptly to an unfamiliar stimulus.

An underlying link between personality traits related to interactions with unfamiliar environments, and the individuals' expectations towards unknown situations might be present, as observed in pigs during a social isolation and novel object task (Asher et al., 2016). The fact that donkeys show different judgement bias, which are not correlated with their life history or living conditions, indicates that optimism/pessimism might not always be a consequence of acute stress or terrible welfare. Instead, it suggests that a trait-like feature might be conditioning these behaviors. It is important to define how these bias affect animals' behavior and cognition, in order to improve our interaction with them and to broaden our notion of animal wellbeing.

5.5.Detour behavior

Results from the U-task suggest that miniature donkeys have cognitive spatial abilities that allow them to detour around a symmetrical and an asymmetric obstacle. The expected strategy to solve the task, for D1 and D2 trials, would be to take the shorter route (i.e. walk around the shorter side of the barrier), yet this was not found to be the general response. Most subjects did not use reasoning and showed no spatial learning during this task, as previously observed in horses (Baragli et al., 2011). Instead, this experiment becomes the second report of lateralized behavior in donkeys (Zucca et al., 2011). One individual revealed the ability to discover new and more expeditious ways to solve the test, as it switched from not having a preferred detour side during the symmetric trials and D1, to choosing the shorter detour during D2, when the asymmetry was highest. It would not be surprising that behavioral lateralization surmounts abstract thought in such a simple task, as it has been reported to be an adaptive strategy for coordination and facilitating action patterns (Ward and Cantalupo, 1997). It is yet to be described how laterality affects donkey cognition, with studies in other species suggesting that it could be matched with impaired reasoning (Baragli et al., 2011), and others proposing that it enables behavioral function in the context of ecology (McGrew and Marchant, 1999) . Although this study found no interactions between Laterality and other variables, it has also been suggested to potentially expose the valence of emotions (Leliveld et al., 2013). The fact that more individuals preferred to detour around the left could be commented on the light of these findings, that indicate a higher involvement of the right hemisphere of the brain in the processing of response to novelty and flight behavior (horses: Austin and Rogers, 2014; Austin and Rogers, 2007), as to food reward and positive social situations (sheep: Reefmann et al., 2009; Versace et al., 2007). As the test required a bucket to be moved in the presence of the animal, flight behavior was common during habituation and not completely eliminated during trials. More specialized tests are required to investigate how a nervous individual would process such a challenge when fear responses are absent, and when compared to a less nervous individual. Accordingly, when Baragli et al. (2011) suggests that non-lateralized horses might have higher spatial reasoning abilities, a question arises on whether these individuals displayed generally higher cognitive skills, or relatively lower fear-related responses, reducing the effects of lateralized processing of cognition. The same arguments apply to the positive correlation found between Concentration scores and the Mean Latency to approach the goal throughout U-task trials, since this trait is also closely related to the processing of daunting situations. The ability to concentrate in the tests required the animals to be relaxed, since potentially frightening stimuli were often present (e.g. noise, cars, unfamiliar human presence) and were the primary object of attention.

5.6.Ethical Note

The present study protocol was approved by the Institutional Animal Care and Use Committee (IACUC; Institutional animal care and use approval #21440). The study subjects were trained and habituated to the study procedures, which were conducted as carefully as possible in order to maximize the scientific output and minimize disturbance to the animals (according to the guidelines of the Association for the Study of Animal Behaviour).

6. Final remarks

Similar studies would benefit from a bigger sample size and longer training/habituation periods, provided that the study subjects are under the same circumstances. Fear levels during the tests were not measured and fear responses to the study apparatus were not completely eliminated before the animals were tested, which could have affected the results. An important improvement to make would be to add controls for arousal, motivation, and distraction confounders (Crump et al, 2018). Given the kinship between the donkeys, a familial link could also be considered in future analysis. Regarding the personality questionnaire, some questions that proved to be highly correlated might have been redundant or prone to ambiguous interpretation by the observers. The same way, it was not perfectly clear what each gradient referred to, in the scale of the described behavior for each question, and the personality factors discriminated do not always correspond to personality traits described in similar studies for other species. Hence, the application of validated behavioral tests with the study subjects would probably have been a more accurate approach to characterize their personalities. Another solution would be to validate a new questionnaire that allowed the observer to characterize donkey personality within the scope of well-studied personality traits that are common to all animal species – facilitating any results to be compared with the results of other studies. Finally, it is important to consider that each individual was only scored in one particular time of their lives, and that the observers did not spend more than a maximum of three hours per day with the animals, on a regular week.

In conclusion, the results reported here indicate that judgement bias might vary between individuals living under the same good conditions, suggesting once more that optimist/pessimism might have a component that is intrinsic to personality. Although not corresponding to the proposed hypothesis, it was shown that specific personality factors might predict the valence of judgement bias. No evidence was found to attribute causality between these and the individual differences in cognition, but unexpected links between personality factors and performance during the cognitive tests were observed. This study represents the second report of judgement bias experiments in donkeys, and the first referring to a possible link between these and personality in this species. The emerging study of intrinsic individual differences in judgement bias - possibly linked to well-known personality traits - is of great importance to understand animal welfare, and to further develop animal behavior studies. Transposable to other species as they likely are, these findings could also yield knowledge about how much cognitive bias experienced by humans are inherent in personality, and independent of circumstances – contributing to the study of human psychology.

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9. Appendix 1

Table 1. The study subjects' information regarding Sex, Age, Housing situation, Life History (referring to their context of arrival at the ranch).

Name	Sex	Age	Housing	Life History
Doris	Female	12 yr	Small pen	Rescued as Adult
Anabelle	Female	12 yr	Big pen	Rescued as Adult
Henry	Male	9 yr	Small pen	Rescued as Adult
Gladys	Female	4 yr	Big pen	Rescued as Adult
Janette	Female	8 yr	Big pen	Rescued as Adult
Lucy	Female	6 yr	Big pen	Rescued as Adult
Nacho	Male	3 yr	Big pen	Rescued as Foal
Robin Hood	Male	3 yr	Big pen	Rescued as Foal
Sofia	Female	7y	Big pen	Rescued as Adult
Storm	Female	1 yr	Big pen	Born at the Ranch
Sunny	Female	1 yr	Big pen	Born at the Ranch
Taquito	Male	3 yr	Big pen	Rescued as Foal

Table 2: Spearman's correlation coefficients (r_s) between questions of the Personality questionnaire (Q1-18). Scores for each question were calculated as the average between all individual scores (mean score of the ten observers). Significant correlations are marked in bold (* : p<0,05; ** : p<0,01).

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Q17	Q18
Q18	**-,660***	,183	,534	,469	**-,695***	,310	-,093	**,651***	**-,616***	,470	-,327	-,168	-,122	,147	,333	,178	,522	**-,589***
Q17	**,852****	**-,620***	-,476	-,129	**,778****	**-,619***	-,214	-,357	**,721****	-,481	,286	,122	,497	**-,602***	**-,675***	-,512	**-,854****	
Q16	**-,756****	**,704***	,492	,232	**-,828****	**,745****	,364	,300	**-,751****	,434	-,454	,175	-,501	,340	**,609***	**,644***		
Q15	**-,595***	,402	,164	,013	-,354	**,862****	,352	-,004	**-,580***	,180	-,280	,190	-,024	,422	,156			
Q14	**-,607***	**,688***	,024	,101	**-,673***	,395	,163	,395	-,308	,329	,000	,000	-,228	,358				
Q13	-,468	,224	,244	,141	-,275	,379	,320	,013	-,179	,192	,331	-,177	-,181					
Q12	,394	-,402	-,396	,083	,484	-,025	-,076	-,259	,321	-,112	,171	,110						
Q11	,109	-,227	-,204	-,174	-,199	,131	-,122	-,383	,181	-,194	-,557							
Q10	,236	-,056	-,325	,137	,491	-,300	,238	,110	,435	-,184								
Q9	-,229	,513	,412	**,676***	-,449	,530	,481	,337	**-,779****									
Q8	**,622***	**-,640***	-,511	-,389	**,631***	**-,766****	-,406	-,451										
Q7	-,576	,366	,022	,206	-,534	,214	,095											
Q6	,023	**,620***	,235	,411	-,133	**,619***												
Q5	-,576	**,676***	,239	,206	-,556													
Q4	**,768****	**-,579***	-,455	-,192														
Q3	,025	,183	,502															
Q2	-,242	,298																
Q1	-,490																	

Table 3: Personality Factors referring to the clustered questions ($-0,5 > r_s > 0,5$). Scores for each individual (calculated as the average between scores from each question included in the Factor).

Personality Factors	Questions Included	Anabelle	Henry	Lucy	Sofia	Storm	Doris	Janette	Gladys	Taquito	Sunny	Nacho	RH
Patience	Q22	7	7	7	7	7	5	7	6	5	7	7	7
Vigilance	Q21	6	6	7	7	8	7	6	7	7	8	7	6
Obedience	Q20	4	4	4	5	4	5	4	5	3	4	4	4
Predictability	Q19	3	5	4	3	5	3	4	3	4	5	3	4
Memory	Q16	7	6	6	6	6	5	7	7	7	7	6	7
Curiosity	Q15	7	8	7	7	8	6	7	8	7	7	8	8
Trainability	Q12	6	7	7	7	6	5	6	7	7	7	7	7
Co-dependency	Q11	7	7	8	7	6	6	7	7	7	6	8	7
Nervousness	Q9, Q13, Q25, Q26, Q27	3	5	4	3	5	6	4	4	5	5	4	3
Friendlyness	Q14, Q17, Q18, Q24, Q25	4	5	5	4	5	5	4	4	5	5	4	4
Concentration	Q10	4	4	4	5	4	4	4	5	4	4	5	5

Table 4: Standard means and deviations for weather variables during the experimental procedures.

	Temperature (°C)	Wind (m/s)	Humidity (%RH)
Memory- F	29,54 ± 3,70	0,92 ± 1,26	26,92 ± 11,36
Memory- IR	18,49 ± 6,71	0,39 ± 0,68	57,63 ± 24,39
Memory- DR	11,15 ± 2,68	0,48 ± 0,74	75,50 ± 14,85
Judgement bias- DL	19,00 ± 5,11	0,48 ± 0,78	49,02 ± 11,00
Judgement bias- T	15,08 ± 4,80	0,64 ± 0,94	55,84 ± 30,86
U-task	19,04 ± 4,13	0,73 ± 0,93	37,27 ± 15,31

10. Appendix 2

Donkey Personality Questionnaire

Name of the person completing the questionnaire:

Date:

Donkey's identification

Name:

Sex:

Age:

Below is a list of behavioral traits and their correspondent description. For each trait a numerical scale is represented, corresponding to the score of that trait's representation in the individual's temperament.

Please take a look at two examples on how to complete this section of the questionnaire:

In the case of a donkey which is often nervous but not always, and has terrible concentration:

Trait	Description	Score
Nervousness	Gets nervous in the presence of noises, insects, etc.	1 Calm 2 3 4 5 6 7 x8 9 10 Nervous
Concentration	Collaborative with the care takers and does not get distracted with the environment.	x1 Not good 2 3 4 5 6 7 8 9 10 Excellent

Please complete the questionnaire according to the previous examples:

Trait	Description	Score									
Nervousness	Gets nervous in the presence of noises, insects, etc.	1 Calm	2	3	4	5	6	7	8	9	10 Nervous
Concentration	Collaborative with the care takers and does not get distracted with the environment.	1 Not good	2	3	4	5	6	7	8	9	10 Excellent
Dependency	Feels comfortable when separated from the herd or when in an unfamiliar environment.	1 Restless	2	3	4	5	6	7	8	9	10 Quiet
Training potential	Easy to train and responds promptly.	1 Not trainable	2	3	4	5	6	7	8	9	10 Very trainable
Excitability	Easily excitable.	1 Not excitable	2	3	4	5	6	7	8	9	10 Very excitable
Friendly towards people	Not aggressive or fearful.	1 Not friendly	2	3	4	5	6	7	8	9	10 Very friendly
Curiosity	Interested in novel objects and usually approaches them.	1 Rarely	2	3	4	5	6	7	8	9	10 Often
Memory	Remembers training or what it learned before.	1 Not good	2	3	4	5	6	7	8	9	10 Excellent
Panic	Reacts exaggeratedly when scared.	1 Never	2	3	4	5	6	7	8	9	10 Frequently

Cooperation	Cooperates with care takers during handling.	1 Never	2	3	4	5	6	7	8	9	10 Always
Emotional stability	Not predictable from one day to the other.	1 Predictable	2	3	4	5	6	7	8	9	10 Unpredicta ble
Obstinacy	Resists to follow an order.	1 Obedient	2	3	4	5	6	7	8	9	10 Stubborn
Vigilance	Alert to its surroundings.	1 Never	2	3	4	5	6	7	8	9	10 Always
Perseverance	Patient with different stimuli.	1 Impatient	2	3	4	5	6	7	8	9	10 Patient
Competitiveness with other donkeys	Dominant in antagonistic encounters with other donkeys.	1 Subordinate	2	3	4	5	6	7	8	9	10 Dominant
Answer to surprise	Easily surprised or scared.	1 Skittish	2	3	4	5	6	7	8	9	10 Composed
Shyness	Shy in new environments.	1 Daring	2	3	4	5	6	7	8	9	10 Shy
Going in and out of the houses	Goes in or out without any problems.	1 Rarely	2	3	4	5	6	7	8	9	10 Always