Developing a 3-Choice Serial Reaction Time Task for Examining Neural and Cognitive Function in an Equine Model

Roberts. Kirsty[1], Hemmings. Andrew.J.[1], McBride. Sebastian.D.[2] & Parker. Matthew.O.[3]

[1]Royal Agricultural University, Stroud Road, Cirencester, Gloucestershire, GL7 6JS

[2] Aberystwyth University, Penglais, Aberystwyth, Ceredigion, SY23 3DA

[3] School of Health Sciences and Social Work, University of Portsmouth, James Watson West building, 2 King Richard 1st Road, Portsmouth, Hampshire, PO1 2FR

Corresponding Author: K. Roberts Tel: 01285 652531. kirsty.roberts@student.rau.ac.uk

Ethics: Ethical approval was provided by the ethics committee and the Royal Agricultural University

Original Research Article

Highlights

- A mobile, fully-automated operant system for the horse is presented
- A validated 3-CSRTT training method has been developed
- Horses required 3 days of training to achieve learning criterion
- Horses reach steady state responding within six sessions
- Horses may provide a complement to rodent models of human neurological dysfunction

Developing a 3-Choice Serial Reaction Time Task for Examining Neural and Cognitive Function in an Equine Model

Roberts. Kirsty[1], Hemmings. Andrew.J.[1], McBride. Sebastian.D.[2] & Parker. Matthew.O.[3]

[1]Royal Agricultural University, Stroud Road, Cirencester, Gloucestershire, GL7 6JS

[2] Aberystwyth University, Penglais, Aberystwyth, Ceredigion, SY23 3DA

[3] School of Health Sciences and Social Work, University of Portsmouth, James Watson West building, 2 King Richard 1st Road, Portsmouth, Hampshire, PO1 2FR

Corresponding Author: K. Roberts Tel: 01285 652531. kirsty.roberts@student.rau.ac.uk

Ethics: Ethical approval was provided by the ethics committee and the Royal Agricultural University

ABSTRACT

Background: Large animal models of human neurological disorders are advantageous compared to rodent models due to their neuroanatomical complexity, longevity and their ability to be maintained in naturalised environments. Some large animal models spontaneously develop behaviours that closely resemble the symptoms of neural and psychiatric disorders. The horse is an example of this; the domestic form of this species consistently develops spontaneous stereotypic behaviours akin to the compulsive and impulsive behaviours observed in human neurological disorders such as Tourette's syndrome. The ability to non-invasively probe normal and abnormal equine brain function through cognitive testing may provide an extremely useful methodological tool to assess brain changes associated with certain human neurological and psychiatric conditions.

New Method: An automated operant system with the ability to present visual and auditory stimuli as well as dispense salient food reward was developed. To validate the system, ten horses were trained and tested using a standard cognitive task (three choice serial reaction time task (3-CSRTT)).

Results: All animals achieved total learning criterion and performed six probe sessions. Learning criterion was met within 16.30±0.79 sessions over a three day period. During six probe sessions, level of performance was maintained at 80.67±0.57% (mean±SEM) accuracy.

Comparison with existing method(s): This is the first mobile fully automated system developed to examine cognitive function in the horse.

Conclusions: A fully-automated operant system for mobile cognitive function of a large animal model has been designed and validated. Horses pose an interesting complementary model to rodents for the examination of human neurological dysfunction.

Highlights

- A mobile, fully-automated operant system for the horse is presented
- A validated 3-CSRTT training method has been developed

- Horses required 3 days of training to achieve learning criterion
- Horses reach steady state responding within six sessions
- Horses may provide a complement to rodent models of human neurological dysfunction

Key words: Horse; Operant; Cognition; Learning; 3-CSRTT; Tourette's Syndrome.

1.0 Introduction

The development of large animal models for examination of neurocognitive dysfunction is increasing in popularity, with several examples in sheep (*Ovis aries domestica;* Morton & Howland, 2013; McBride *et al.,* 2016) and pigs (*Sus scrofula domesticus;* Morton & Howland, 2013). Large animal and human brains demonstrate distinct neuroanatomical similarities which rodent models lack, for example both possess gyrencephalic cerebral cortex and heterogeneous striatal structures (McBride & Hemmings, 2005; Morton & Howland, 2013); the latter being particularly important for detailed study of basal ganglia disorders such as Tourette's syndrome (TS; Worbe *et al.,* 2012), Parkinson's disease (PD; Obeso *et al.,* 2000), addiction (Haber, 2003) and early onset Huntington's disease (HD; Rosenblatt & Leroi, 2000). Whilst relatively expensive compared to rodents, large animals can be housed in a naturalised environment (Morton & Howland, 2013), negating the influence of artificial, sub-optimal conditions that can themselves lead to neural dysregulation (Garner & Mason, 2002). Furthermore the extended life expectancy of the large animals at over 15 years compared to the rodent at approximately 2 years (Levine, 1997; Morton & Howland, 2013), is also highly beneficial for modelling slow progression neurodegenerative diseases including PD and HD, but also phasic pathology such as schizophrenia as well as further aiding our understanding of the longitudinal development of TS.

One potentially advantageous large animal model of neurological dysfunction that is yet to be explored is the horse (*Equus caballus*). As well as having all of the large animal model advantages previously described, a percentage of horses also consistently develop a hyperdopaminergic state associated with spontaneous stereotypy development (McBride & Hemmings, 2005). These behaviours have morphological and behavioural characteristics that are extremely similar to the spontaneous compulsive/impulsive symptoms of neurological disorders such as TS. It may be extremely useful therefore, to be able to probe equine brain function in order to gain a further understanding of the neurological changes associated with human neurological and psychiatric conditions that manifest stereotypy as a symptom. This can be achieved both invasively (neurophysiological assays) and non-invasively (cognitive battery), but the latter has the advantage of being able to monitor the animal over time within a longitudinal context.

The horse is able to achieve many of the standard cognitive paradigms used in neurological testing, such as extinction learning tasks (Hemmings *et al.,* 2007; Roberts *et al.,* 2015), the two-choice discrimination reversal task (Hanggi & Ingersoll, 2009), the match-to-sample task (Gabor & Gerken, 2012), and memory tests (Hanggi & Ingersoll, 2009), amongst others (Hanggi, 2003; Hanggi, 2005). Overall these data strongly suggest that the horse may be a highly suitable large animal model of human neurological and psychiatric disorders.

The primary objective of this study, therefore, was to design and produce a portable, fully-automated operant system (OS) designed for efficient, simple cognitive and neural testing for the horse. In order to validate the OS, a three choice version of the well utilised five choice serial reaction time task in other species (Bari *et al.,* 2008; Parker *et al.,* 2012) was developed and tested using 10 horses.

2.0 System Specification and Fabrication

2.1 System Specification

A number of key aspects for the development of the OS were important. The portability of the OS was crucial, both for short-term use, but also for the potential long-term cognitive testing at different establishments. This required the OS to be light and small enough for ease of transport. It was also critical that the OS was sufficiently robust to protect it from damage under standard usage conditions.

Ethological relevance of the OS was also considered to ensure the horse would be both capable and motivated to perform the cognitive test. Some evidence indicates that presenting the stimuli at ground level is more suitable for horses (Hall *et al.,* 2003). However, complex cognitive tasks could involve the presentation of picture based stimuli e.g. shapes, which may require greater visual acuity, and therefore the use of the binocular vision (Harman *et al.,* 1999). Binocular vision is suggested to cover approximately 80° to the front of the horse and is typically used when the head is raised, with lateral vision utilised when the head is closer to the ground (Harman *et al.,* 1999). As such, visual acuity may be less clear when the horse is observing at ground level (Hall, 2007), therefore the long term use of the OS for a range of cognitive tests could be compromised should the stimuli be presented at ground level. Additionally, the feed trough was designed to sit below the screens to which small amounts of fibre based concentrate feed were to be deposited. Whilst primarily grazing animals (Hall, 2007), horses can often be observed foraging at wither-to-head level in shrubs and trees (van den Berg *et al.,* 2015), and are similarly fed in the stable environment with a hay net under some management regimes (Ellis *et al.,* 2015). Thus chest height feeding apparatus was deemed suitable in this case. As horses are trickle feeders they do not need to be food deprived prior to the performance of the cognitive task in order to maintain motivation (Parker *et al.,* 2008), increasing the ethological relevance with regards to the release of small amounts of concentrate feed as reward, as well as minimising ethical concerns associated with enforced feed restriction recommended to maintain motivation during the rodent 5-choice serial reaction time task (Bari *et al.,* 2008).

In addition to the visual stimuli, different auditory cues were utilised to alert the horse as to the imminent display of the visual stimulus, as well as for timeout, correct and incorrect choice selection. Horses are able to recognise the vocal calls of a ‘familiar’ compared to ‘stranger’ horse (Basile *et al.,* 2009). Additionally, horses have been shown to differentiate between a ‘pleasant’ soft tone of human voice compared to a ‘stern’ human voice, altering their behaviour accordingly (Merkies *et al.,* 2013). This would indicate therefore that the horse can identify between different auditory stimuli and use this as a basis to guide behaviour. Furthermore, the use of visual and auditory cues when presented separately has been shown to hold the attention of the horse towards the stimuli (Christensen *et al.,* 2005); a phenomenon which would be of value here to ensure the animal is actively partaking in the cognitive task.

The removal of human interference on horse performance was also an important aspect of design. The famous case of ‘Clever Hans’ is an excellent example of the horse being able to utilise subtle human cues to effectively perform the task, despite not actually learning the specific rules (Lambert, 1999). More recent research has also utilised this ability to cue the horse to guide the horse towards the food reward (Lovrovich *et al.,* 2015). It is evident then that even small cues by any human observer could significantly impact horse performance on cognitive tasks, however unknowingly. Thus, during experimentation the role of the human was limited to positioning the horse in front of the OS, maintaining horse and human safety at all times by removing the animal should dangerous behaviours be performed, and the removal of the OS following task completion.

To ensure the long-term use of the OS, the paradigm logic was processed using Matlab R2014a (Math-works, UK) with the Psychtoolbox (Psychtoolbox.org) addition. With the use of 12bit data acquisition device (DAQ; USB-1208fs, Measurement Computing, Norton, USA) Matlab software relays information from input channels i.e. the infrared sensors towards the output channels. For example, if the Matlab logic determines that a response was correct based on the information of sensor origin in combination with cognitive task coding, then a transitor-transitor logic (TTL) pulse is sent via the DAQ to the feed-dispenser which then releases the reward. In contrast, if an inappropriate sensor is selected, Matlab will interpret this as such and no pulse will be sent and a different outcome will result. This process for the 3-CSRTT can be overviewed in Fig. 1, in line with the horse action.

2.2 System Fabrication

The OS was designed to be fully portable, but also to be mountable within the animal's individual stable (Fig. 2). A previously successful set-up conducted with sheep (McBride *et al.,* 2016) was utilised as a basis for the design, though was adapted significantly to address the OS requirements. Visual stimuli were presented utilising a three LCD screen (ePathChina, China) format with partitions between screens. The partitions were important to prevent the horse from moving its head between the three screens, thereby activating multiple infrared sensors (Omron, Nufringen, Germany), but also to ensure the horse made a discrete screen selection. Responses were registered once the infrared beam located above each screen was broken by the horses muzzle making contact with the screen. To account for sensor sensitivity, three positions at 20, 45 and 70mm from the backboard were available at the sensor mounting apparatus, allowing the sensors to be moved depending on the sensitivity required from the task. If the 'correct' response was made by the horse and registered with the breaking of the infrared beam, a TTL signal was sent to the DAQ, which triggered the release of 5g of unbranded horse and pony nuts (Jollyes, UK) from the feed-dispenser. The feed-dispenser design was unchanged from the sheep version (McBride *et al.,* 2016) with the exception of adapting to 6.50mm horse feed. As such, the feed-dispenser was operated from a direct power source (24 V), as opposed to mains electricity. The feed was released into a feed trough, though in contrast to the ovine model, the horse set-up had one trough positioned central to the backboard. For portability purposes, the feed trough was removable during transit and could be repositioned on-site.

To mount the OS onto a stable door, a brace structure attached to the backboard on-site via a series of boltholes located to the sides of the OS was constructed (Fig. 2). Ten paired boltholes with 12mm spacing allowed the OS to be adapted in height dependent on the size of the horse, where the screens were positioned slightly below eye level (Brubaker & Udell, 2016), accounting for the blind spot in directly in front of the horse. The OS, including the feed-dispenser was custom-built (Quality Equipment, Woolpit, UK). To ensure full portability, the OS could run off of mains powered electricity, or via a 12v car battery (Halfords, UK) with the use of an electric inverter (Photonic Universe Ltd, UK). Additionally, the feed-dispenser was designed run off of a chargeable internal battery (McBride *et al.,* 2016). The entire operation of the OS was accomplished utilising the TOUGHPAD FZ-G1 (Panasonic, UK), which allowed the programming of Matlab, on-site running of the code, and recording outputs. The final weight of the OS was approximately 32kg, and therefore required two humans to safely lift the OS into position on the stable door.

3.0 Behavioural Testing

3.1 Sample Animals

Ten experimentally naïve horses aged 2-17 years, of various breeds and sex (n=5 mares; n=5 geldings) ranging from 12-16.2 hands high (hh) volunteered by private owners were recruited. All animals were fed on a forage based diet (e.g. hay/haylage). No management or feeding regime modifications were implemented, given that feed deprivation is thought to not be required to maintain horses feeding motivation as trickle feeders (Parker *et al.,* 2008). The experimental procedure was conducted within the horses' own stable, and the animal was not limited in terms of eye-contact with conspecifics throughout in an attempt to reduce stress

3.2 Pavlovian Acclimation

The horse was loosely held by the operator for safety reasons, who was positioned on the right and side of the device though in such a manner as to be unaware of any screen presentations, but also to initiate the software. The operator initiated the Pavlovian Acclimation stage of training via the TOUGHPAD. All three LCD screens remained black, with 5g feed released into the trough every 30 seconds for 15 trials. Following the final trial, a long low-pitched auditory tone (AT; 260Hz sinusoidal wave form, 1.9s) was given to signify the end of the session.

3.3 Pre-Training 1

The purpose of Pre-Training 1 (PT1) was to expose and condition the animal to associate both the audio conditioned stimulus (CS1; 400Hz sinusoidal wave form, 0.15s) and visual conditioned stimulus (CS2; a plain white screen) with feed reward. The session commenced with the concurrent presentation of CS1 and CS2 on all three screens. If an operant response was conducted on any of the three screens within a 30 second time-limit, 5g of feed was immediately released. If no operant response was sensed, feed was released after 30 seconds. Following feed release, CS2 was removed to provide black screens only for a 15 second inter-trial-interval (ITI) to allow the animal to consume the feed prior to the initiation of the next trial. As with all sessions, a total of 15 trials was utilised. An AT to signify the end of the session was presented after the final trial. Learning criterion to proceed to Pre-Training 2 was set at one session with at least a 50% response rate towards CS2.

3.4 Pre-Training 2

Pre-Training Stage 2 (PT2) was designed to further encourage an operant response from the animal, and extend trial and error learning initiated during PT1. Similar to PT1, PT2 was initiated by the simultaneous presentation of CS1 and CS2 on all three screens. CS2 was presented for 30 seconds, however if zero operant responses were performed during this time, the 15 second ITI commenced with no feed release. If an operant response was sensed on any of the three screens, 5g of feed was immediately released from the feed hopper followed by the 15 second ITI. Following the final trial, the end of the session was signalled by AT presentation. Learning criterion to proceed onto Pre-Training 3 was set at one session with a minimum of 50% response rate.

3.5 Pre-Training 3

The purpose of Pre-Training 3 (PT3) was to introduce the horse to CS2 on one screen, and to encourage the horse to respond only towards this screen. To initiate the session, CS1 is given concurrently with CS2 on all three screens. The horse was required to select any one of the three screens to achieve a feed reward and commence the remainder of the session. Following a 15 second ITI, CS1 was once more presented simultaneously with CS2, though importantly CS2 occurred on only one of the three screens in a pseudorandom order. Each screen presented CS2 five times. To continue onto the next trial within the session, the horse was required to select the screen displaying CS2. There was no time-limit to perform this operant response. Once an operant response was conducted on the correct screen, 5g of feed was released and the 15 second ITI was initiated prior to the next trial within the sequence. To complete the session, the animal was required to select the correct screen for each of the 15 trials. The end of the session was signified by the AT. Every horse was required to perform three sessions of PT3 before commencing Pre-Training 4a.

3.6 Pre-Training 4a

The aims of Pre-Training 4a (PT4a) were two-fold:- first, to introduce the consequences of error to the horse; and second, to introduce a time-limit within which an operant response was required. As with PT3, the session initiation was highlighted with the presentation of CS1 and CS2 on all three screens concurrently. To initiate the session, the horse was required to perform an operant response on any of the three screens to achieve a feed reward and initiate the 15 second ITI. No time-limit was given to perform this initial operant response. Following the 15 second ITI, both CS1 and CS2 were presented, with CS2 occurring on only one screen in a pseudorandom order, each screen displaying CS2 five times within the session. During PT4a, an incorrect operant response (error of commission) resulted in a short high-pitched incorrect auditory tone (IAT; 1000Hz sinusoidal wave form, 0.5s) and immediate ITI commencement with no feed reward attainment. CS2 during PT4a was only displayed for 20 seconds, with an additional 5 second black screened limited hold (LH), allowing a maximum 25 seconds for the horse to perform an operant response. If no operant response was performed during this time (error of omission), a time-out auditory tone (TOAT; 2250Hz sinusoidal wave form, 0.3s) was produced, and the ITI commenced with no feed reward attainment. Should the horse perform an operant response on the correct screen, immediate 5g of feed delivery and ITI commencement was the result. The end of the session was highlighted by the AT. To proceed to Pre-Training 4b, the horse was required to perform two consecutive sessions of PT4a with a minimum 80% accuracy, inclusive of omitted responses. The probability of such events occurring by chance was calculated at $p = 5.21^{-8}$ utilising a binomial probability calculation. Any compulsive choices (i.e. those made during the ITI) were recorded.

3.7 Pre-Training 4b

Pre-Training 4b (PT4b) was conducted in the same manner as PT4a, except that the time-limit for which to perform the operant response was reduced. During PT4b, CS2 was presented for 10 seconds, with a 5 second LH. Thus the time allowed to perform an operant response during PT4b was reduced to 15 seconds. The learning criterion to proceed to Pre-Training 4c was set at two consecutive sessions of PT4b with a minimum 80% accuracy ($p = 5.21^{-8}$) inclusive of omitted responses. Once more compulsive choices were monitored.

3.8 Pre-Training 4c

Pre-Training 4c (PT4c) was a further variation of PT4a and PT4b, with the aim to reduce the response rate to its final duration. For PT4c, CS2 was presented for 5 seconds, followed by a 5 second LH before time-out. The horse therefore had a total of 10 seconds to complete an operant response. To proceed onto Task Training, the horse was required to achieve learning criterion of two consecutive sessions with a minimum 80% accuracy ($p = 5.21^{-8}$) inclusive of omitted responses. Compulsive responses were monitored though unpunished.

3.9 Task Training

Task Training (TT) was utilised for the introduction of the pre-stimulus interval (PSI), during which impulsive choices towards screens could be conducted. The session was initiated as per PT4c. Following the ITI, CS1 was presented, with CS2 being displayed 5 seconds later on one screen in a pseudorandom order for 5 seconds, with a 5 second LH. Each screen displayed CS2 five times throughout the session. If an operant response was performed during the 5 second PSI this was counted as an impulsive error, resulting in IAT, zero feed attainment and immediate cessation of that trial, with the commencement of the ITI. If no impulsive choice was made, the trial continued. Correct choices within the time-limit were reinforced with 5g of feed, incorrect responses were highlighted with IAT followed immediately by the ITI. Should no response be made within the time-limit, this resulted in TOAT followed by the ITI. Learning criterion was set at two consecutive TT with a minimum 80% accuracy (p = 5.21^{-8}) inclusive of omitted and impulsive responses. Compulsive responses were monitored though unpunished. Should the horse not achieve learning criterion within a maximum of ten sessions, this was noted training ceased. During any phase, should the horse cease responding sessions for that day were halted, and repeated the following day. This was not however an issue in this current study. Should overall learning criterion not be met, then this would demonstrate that ten sessions is not sufficient for the horse to learn the 3-CSRTT and adaptions required to be made. Day criterion were also set in an attempt to standardise overnight learning consolidation. Day 1 training ended following two PT4a sessions, Day 2 ceased following two TT sessions, and Day 3 training concluded once learning criterion had been met, followed by six probe sessions.

3.10 Analysis

To determine the success of the OS and its corresponding coding a number of measurements were taken, including sessions to criterion, session duration, latency of approach and compulsive/impulsive responses where appropriate. From these data, the mean duration, sessions and days required to meet overall and individual task phase learning criteria could be calculated. To examine whether post-criterion performance were stable, data were subject to a repeated measures ANOVA. These data will then provide a standardised procedure for application of the equine 3-CSRTT.

4.0 Results

All animals successfully attained learning criterion (Table 1). The total number of sessions (mean±SEM) required to achieve learning criterion was 16.30±0.79 over three consecutive days. On Day 1 8.00±0.00 (mean ±SEM) sessions were completed, compared to 7.40±0.74 sessions on Day 2 and 0.90±0.36 training sessions on Day 3. Fewer sessions were required on Day 3, due to a number of horses already achieving total learning criterion on Day 2. On Day 3, the six probe sessions were also completed (Table 2).

The one-way repeated measures ANOVA determined that there was no significant difference in percentage accuracy of performance ($F(5, 45) = 0.164$, $p=0.974$) over the six probe sessions. No other significant differences were observed for the remaining parameters (Table 2).

5.0 Discussion

In this paper we have introduced a fully portable OS for horses. The design of the OS enabled ease of set up within the horses' stable by a lone operator. Whilst a 3-CSRTT is tested here, the use of Matlab programming demonstrates that the OS could simply adapted to conduct a series of other operant tests, for example stop-signal reaction time tasks, paired associates learning etc. subject to suitable coding development. Such adaptations of the OS provided here could therefore provide enhanced understanding of a variety of neural circuits in the horse for human comparison.

Horses required a total of 16.30±0.79 sessions to attain learning criterion. Stable performance was maintained at 80.67±0.57% (mean±SEM) accuracy during the six probe trials. Additional non-significant differences over the six probe sessions for all parameters (Table 2) also indicated that the animals had efficaciously learned the task, and reached steady state performance. The success rate to achieve learning criterion was excellent at 100%. This indicates that the horse successfully learned the 3-CSRTT and would therefore provide a suitable complementary model for investigating attentional, impulsive and compulsive responding; behaviours synonymous with stereotypy performance. Such parameters may prove useful in the continuing understanding of TS. Indeed, horses which perform the oral stereotypy crib-biting demonstrate a tendency to conduct impulsive decisions in a reinforcer choice procedure (Parker *et al.,* 2008), and also demonstrate apparent lack of sensitivity to extinction learning (Hemmings *et al.,* 2007; Roberts *et al.,* 2015). This behavioural manifestation is thought to arise from midbrain dopaminergic dysfunction (McBride & Hemmings, 2005). Thus, crib-biting horses may have an impulsive/compulsive phenotype similar to TS, highlighting the advantage of using the horse as an improved large animal model. Further application could apply to other neurological disorders as the horse also spontaneously develop the degenerative disorder pituitary pars intermedia dysfunction (PPID; Gehlen *et al.,* 2014) which may provide an interesting model for human neurodegenerative disease such as PD.

Rodent models require approximately 25 sessions to reach learning criterion; though it is of note that rodent models perform a five choice paradigm, and also employ 0.5s stimulus duration, thus whilst not directly comparable demonstrates the potential of our equine OS for further validation. Additionally, rodent sessions comprise of significantly more trials per session; 100 compared to the 15 utilised for the horse here (Bari *et al.,* 2008; Winstanley *et al.,* 2008). The decision to utilise a lower 15 trials/session for the horse was based largely on previous sheep operant training (McBride *et al.,* 2016), given that both equine and ovine are quadruped ungulate species. Furthermore, previous pilot study in our laboratory indicated that sessions of 45 trials resulted in a decrease in motivation to perform the task; a finding not observed during shorter sessions trialled here. Also of note is that in rodent (Bari *et al.,* 2008; Winstanley *et al.,* 2008) and fish (Parker *et al.,* 2012) versions, each animal only performs one session per day. This may well result from the larger number of trials per session utilised for these animals. Due to the stable motivation and responding of the horses during both this study and earlier pilot work, it was apparent that the horse was capable of performing a much larger number of sessions per day. Whilst this may seem large in comparison to rodent and fish work, this essentially brings the number of individual trials performed in line with the recommended rodent procedure (Bari *et al.,* 2008). Additionally horses are known trickle feeders, having evolved as a grazing and browsing animal, and at pasture horses can graze for up to 18 hours a day (Thorne *et al.,* 2005). Thus achieving a small amount of fibrous concentrate feed repeatedly could be construed as ethologically relevant in the context of natural horse feeding behaviours. It is possibly because of this elevated feeding capacity and grazing type behaviour evolved by horses that these animals here were able to continue for a greater number of sessions daily as opposed to rodent and fish equivalents. A similar reason could also account for the maintained motivation also observed by the horses in this study.

Each horse was required to reach a specific level of learning in terms of learning criterion achieved per day as opposed to a specific number of sessions. The primary reason for this was as an attempt to standardise the level of learning and memory consolidation which was likely to occur overnight (Williams *et al.,* 2008) through paradoxical sleep processes (Pederson *et al.,* 2004; Marshall & Born, 2007; Diekelmann & Born, 2010). Given that the motivation to perform the task remained high, limiting the number of sessions per day could potentially be detrimental to learning and task performance parameters by increasing frustration around the OS due to premature removal, in line with anticipation frustration theory (Amsel, 1992). However, standardisation of such tasks is important to stabilise learning and ensure the same training regime for each individual. As such, the protocol suggested here provides a standardised training regime for the 3-CSRTT by ensuring every horse is reaching a specific level of performance each day. This also allows additional learning parameters to be measured, for example number of sessions required to reach learning criterion per stage, but also through number of sessions required to be performed daily to attain these. Such parameters may well provide important information when investigating differences between behavioural phenotypes.

The design of the OS allows for supplementary adaptations to be made to our baseline code for the 3-CSRTT. For example, it would be interesting to determine whether shortening the reaction time and limited hold duration to that more in line with the rodent work (Bari *et al.,* 2008) would influence the attentional or impulsive/compulsive responding of the horse. Additionally, rodent and fish models recommend a variety of adaptations to further probe the neural circuitry. Such examples include the use of a variable ITI (both long and short), adjusting the brightness of the visual stimuli, and the introduction of a distracting noise (Bari *et al.,* 2008). Indeed, the further development of the equine OS is ongoing in our laboratory, leading to enhanced adaptability of our OS.

6.0 Conclusion

Here a fully automated OS allowing the portable cognitive testing has been developed and successfully tested with a protocol produced to allow standardised replication of the 3-CSRTT. Total learning criterion was achieved by 100% horses over three days, in 16.30±0.79 sessions. The OS developed here has excellent potential for further application as a result of flexible design and use of Matlab programming, for both 3-CSRTT but also for a variety of other cognitive tests following suitable coding and testing. As such, the application of this OS could initiate the utilisation of the horse as a suitable large animal model for the examination of dopaminergic conditions, including TS in a natural setting.

Acknowledgements

The authors would like to thank the Royal Agricultural University Research Committee for the funding of this research as part of a PhD project at the Royal Agricultural University.

References

Amsel, A. (1992) Frustration theory: Many years later. *Psychological bulletin*. 112(3), 396.

Bari, A., Dalley, J. W. & Robbins, T. W. (2008) The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. *Nature protocols*. 3(5), 759-767.

Basile, M., Boivin, S., Boutin, A., Blois-Heulin, C., Hausberger, M. & Lemasson, A. (2009). Socially dependent auditory laterality in domestic horses (Equus caballus). *Animal cognition*. 12(4), 611-619.

Brubaker, L. & Udell, M. A. (2016) Cognition and learning in horses (*Equus caballus*): What we know and why we should ask more. *Behavioural processes*. 126, 121-131.

Christensen, J. W., Keeling, L. J. & Nielsen, B. L. (2005) Responses of horses to novel visual, olfactory and auditory stimuli. *Applied Animal Behaviour Science*. 93(1), 53-65.

Diekelmann, S. & Born, J. (2010) The memory function of sleep. *Nature Reviews Neuroscience*. 11(2), 114-126.

Ellis, A.D., Fell, M., Luck, K., Gill, L., Owen, H., Briars, H., Barfoot, C. & Harris, P. (2015) Effect of forage presentation on feed intake behaviour in stabled horses. *Applied Animal Behaviour Science*. 165, 88-94.

Gabor, V. & Gerken, M. (2012) Cognitive testing in horses using a computer based apparatus. *Applied Animal Behaviour Science*. 139(3), 242-250.

Garner, J. P. & Mason, G. J. (2002) Evidence for a relationship between cage stereotypies and behavioural disinhibition in laboratory rodents. *Behavioural brain research*. 136(1), 83-92.

Gehlen, H., May, A. & Bradaric, Z. (2014) Comparison of Insulin and Glucose Metabolism in Horses with Pituitary Pars Intermedia Dysfunction Treated Versus Not Treated with Pergolide. *Journal of Equine Veterinary Science*. 34(4), 508-513.

Haber, S. N. (2003) The primate basal ganglia: parallel and integrative networks. *Journal of chemical neuroanatomy*. 26(4), 317-330.

Hall, C. (2007) The impact of visual perception on equine learning. *Behavioural processes*. 76(1), 29-33.

Hall, C. A., Cassaday, H. J. & Derrington, A. M. (2003) The effect of stimulus height on visual discrimination in horses. *Journal of animal science*, 81(7), 1715-1720.

Hanggi, E. B. & Ingersoll, J. F. (2009) Long-term memory for categories and concepts in horses (*Equus caballus*). *Animal cognition*. 12(3), 451-462.

Hanggi, E. B. (2003) Discrimination learning based on relative size concepts in horses (*Equus caballus*). *Applied Animal Behaviour Science*. 83(3), 201-213.

Hanggi, E. B. (2005, December). The thinking horse: cognition and perception reviewed. In: *AAEP Proceedings* (Vol. 51, pp. 246-255).

Harman, A. M., Moore, S., Hoskins, R. & Keller, P. (1999) Horse vision and an explanation for the visual behaviour originally explained by the ‘ramp retina’. *Equine veterinary journal*. 31(5), 384-390.

Hemmings, A., McBride, S. D. & Hale, C. E. (2007) Perseverative responding and the aetiology of equine oral stereotypy. *Applied Animal Behaviour Science*. 104(1), 143-150.

Lambert, M. J. (1999) Are differential treatment effects inflated by researcher therapy allegiance? Could clever Hans count? *Clinical Psychology: Science and Practice*. 6(1), 127-130.

Levine, H. J. (1997) Rest heart rate and life expectancy. *Journal of the American College of Cardiology*. 30(4), 1104-1106.

Lovrovich, P., Sighieri, C. & Baragli, P. (2015) Following human-given cues or not? Horses (Equus caballus) get smarter and change strategy in a delayed three choice task. *Applied Animal Behaviour Science.* 166, 80-88.

Marshall, L. & Born, J. (2007) The contribution of sleep to hippocampus-dependent memory consolidation. *Trends in cognitive sciences*. 11(10), 442-450.

McBride, S. D. & Hemmings, A. (2005) Altered mesoaccumbens and nigro-striatal dopamine physiology is associated with stereotypy development in a non-rodent species. *Behavioural brain research*. 159(1), 113-118.

McBride, S. D., Perentos, N. & Morton, A. J. (2016) A mobile, high-throughput semi-automated system for testing cognition in large non-primate animal models of Huntington disease. *Journal of neuroscience methods*. 265, 25-33.

Merkies, K., MacGregor, H., Ouimette, M., Bogart, E. & Miraglia, K. (2013). Does the human voice have a calming effect on horses? *Journal of Equine Veterinary Science*. 33(5), 368.

Morton, A. J. & Howland, D. S. (2013) Large genetic animal models of Huntington's disease. *Journal of Huntington's disease*. 2(1), 3-19.

Obeso, J. A., Rodriguez-Oroz, M. C., Rodriguez, M., Lanciego, J. L., Artieda, J., Gonzalo, N. & Olanow, C. W. (2000) Pathophysiology of the basal ganglia in Parkinson's disease. *Trends in neurosciences*. 23, S8-S19.

Parker, M. O., Millington, M. E., Combe, F. J. & Brennan, C. H. (2012) Development and implementation of a three-choice serial reaction time task for zebrafish (*Danio rerio*). *Behavioural brain research*. 227(1), 73-80.

Parker, M., Redhead, E. S., Goodwin, D. & McBride, S. D. (2008) Impaired instrumental choice in crib-biting horses (*Equus caballus*). *Behavioural brain research*. 191(1), 137-140.

Pedersen, G. R., Søndergaard, E. & Ladewig, J. (2004) The influence of bedding on the time horses spend recumbent. *Journal of Equine Veterinary Science*. 24(4), 153-158.

Roberts, K., Hemmings, A., Moore-Colyer, M. & Hale, C. (2015) Cognitive differences in horses performing locomotor versus oral stereotypic behaviour. *Applied Animal Behaviour Science*. 168, 37-44.

Rosenblatt, A. & Leroi, I. (2000) Neuropsychiatry of Huntington’s disease and other basal ganglia disorders. *Psychosomatics*. 41(1), 24-30.

Thorne, J. B., Goodwin, D., Kennedy, M. J., Davidson, H. P. B. & Harris, P. (2005). Foraging enrichment for individually housed horses: practicality and effects on behaviour. *Applied Animal Behaviour Science*. 94(1), 149-164.

van den Berg, M., Brown, W. Y., Lee, C. & Hinch, G. N. (2015) Browse-related behaviors of pastured horses in Australia: A survey. *Journal of Veterinary Behavior: Clinical Applications and Research*. 10(1), 48-53.

Williams, D.C., Aleman, M., Holliday, T.A., Fletcher, D.J., Tharp, B., Kass, P.H., Steffey, E.P. & Lecouteur, R.A. (2008) Qualitative and quantitative characteristics of the electroencephalogram in normal horses during spontaneous drowsiness and sleep. *Journal of veterinary internal medicine*. 22(3), 630-638.

Winstanley, C. A., Chudasama, Y., Dalley, J. W., Theobald, D. E., Glennon, J. C. & Robbins, T. W. (2003) Intra-prefrontal 8-OH-DPAT and M100907 improve visuospatial attention and decrease impulsivity on the five-choice serial reaction time task in rats. *Psychopharmacology*. 167(3), 304-314.

Worbe, Y., Malherbe, C., Hartmann, A., Pélégrini-Issac, M., Messé, A., Vidailhet, M., Lehéricy, S. & Benali, H. (2012) Functional immaturity of cortico-basal ganglia networks in Gilles de la Tourette syndrome. *Brain*. 135(6), 1937-1946.

Table

Session	Training Day	Sessions Required	Session Duration (s)	Compulsive Responses	Impulsive Response (%)
PA	1	1.00±0.00	497.94±0.03	-	-
PT1	1	1.00±0.00	477.85±22.47	-	-
PT2	1	1.00±0.00	446.68±18.82	-	-
PT3	1	3.00±0.00	520.12±40.12	-	-
PT4a	2	3.20±0.70	426±19.56	2.77±0.91	-
PT4b	2	2.20±0.13	378.83±11.88	2.02±0.78	-
PT4c	2/3	2.00±0.00	349.69±6.56	2.65±1.18	-
TT	3	2.90±0.38	411.14±6.35	4.73±1.35	5.77±1.62

Table 1. Sessions and corresponding parameters required to achieve learning criterion (mean±SEM)

Table

Table 2. Parameters recorded from the six probe sessions (mean±SEM). NB – all *p* values are obtained from repeated measures ANOVA. [a] denotes sphericity was not violated, [b] denotes sphericity was violated and Greenhouse-Geisser *p* value was used

Parameter	**1**	**2**	**3**	**4**	**5**	**6**	***p* value**
Duration (s)	418.86±10.25	404.74±7.24	415.14±6.67	410.44±7.67	411.96±9.71	415.23±9.24	0.278[a]
Response Latency (s)	3.71±0.45	3.30±0.30	3.63±0.32	3.40±0.37	3.16±0.37	3.63±0.42	0.326[b]
Accuracy (%)	80.00±4.77	79.33±3.78	82.67±4.24	80.67±3.51	79.33±7.98	82.00±4.22	0.974[a]
Commission Errors (%)	6.00±1.85	9.33±3.47	6.67±2.43	12.00±2.77	6.67±2.63	8.00±1.94	0.400[b]
Impulsive Responses (%)	8.67±2.44	7.34±3.36	3.34±1.11	3.34±0.37	4.00±1.47	2.00±1.02	0.133[b]
Impulsive Response Latency (s)	2.63±0.43	3.50±0.46	2.38±0.53	2.84±0.46	3.02±0.27	1.87±0.50	0.241[b]
Compulsive Responses	3.80±0.68	3.10±0.82	2.30±0.78	4.40±1.11	2.90±0.85	2.30±0.79	0.314[a]
Compulsive Response Latency (s)	6.33±1.07	4.47±1.18	6.19±1.12	6.05±1.15	6.80±1.74	7.04±1.50	0.677[b]
Omission (%)	6.00±3.64	4.00±3.33	7.33±3.06	4.00±2.27	10.00±7.32	8.00±4.53	0.427[b]

Figure

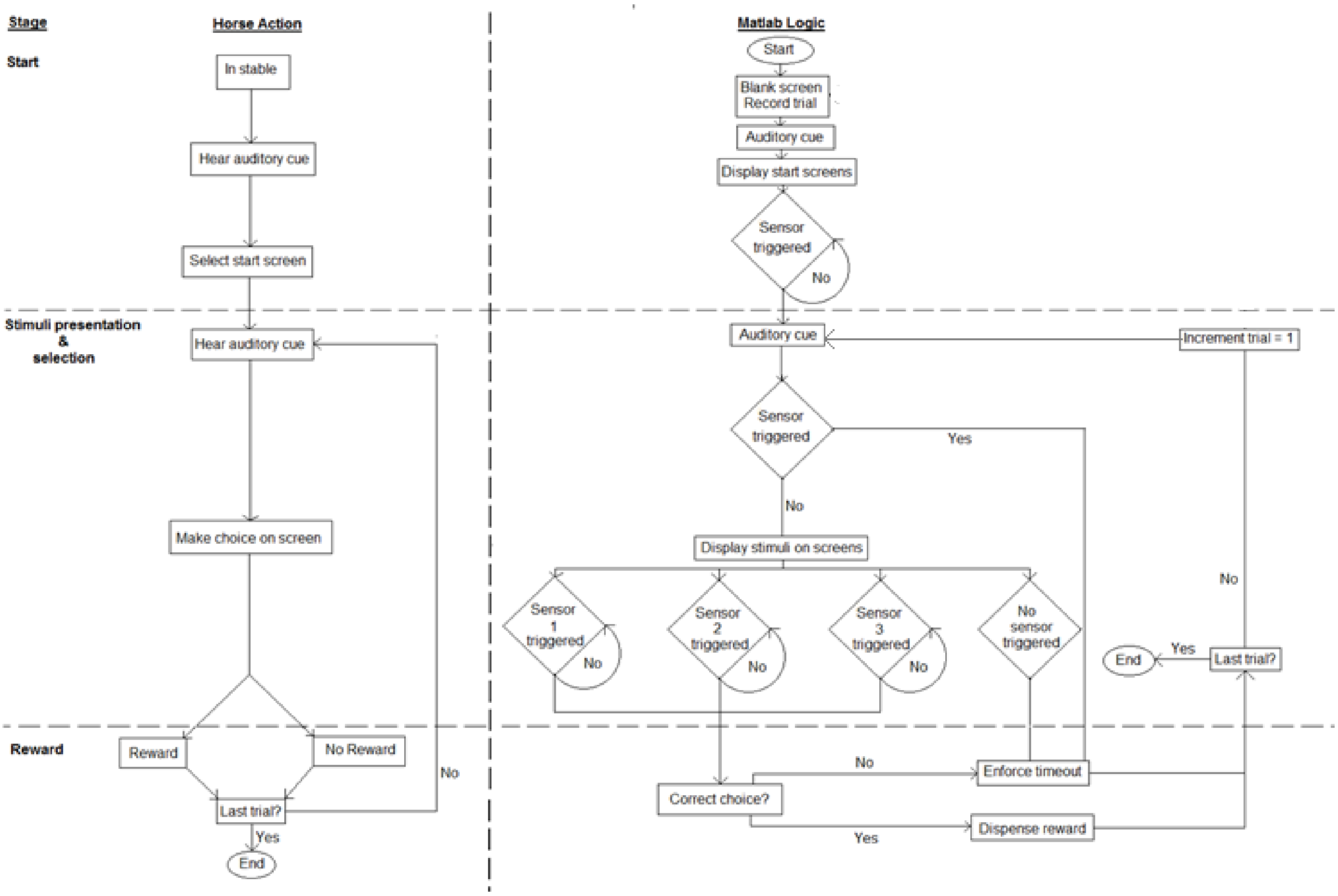

Figure 1. The Matlab logic in relation to horse action during the 3-CSRTT (adapted from McBride *et al.,* 2016)

Figure

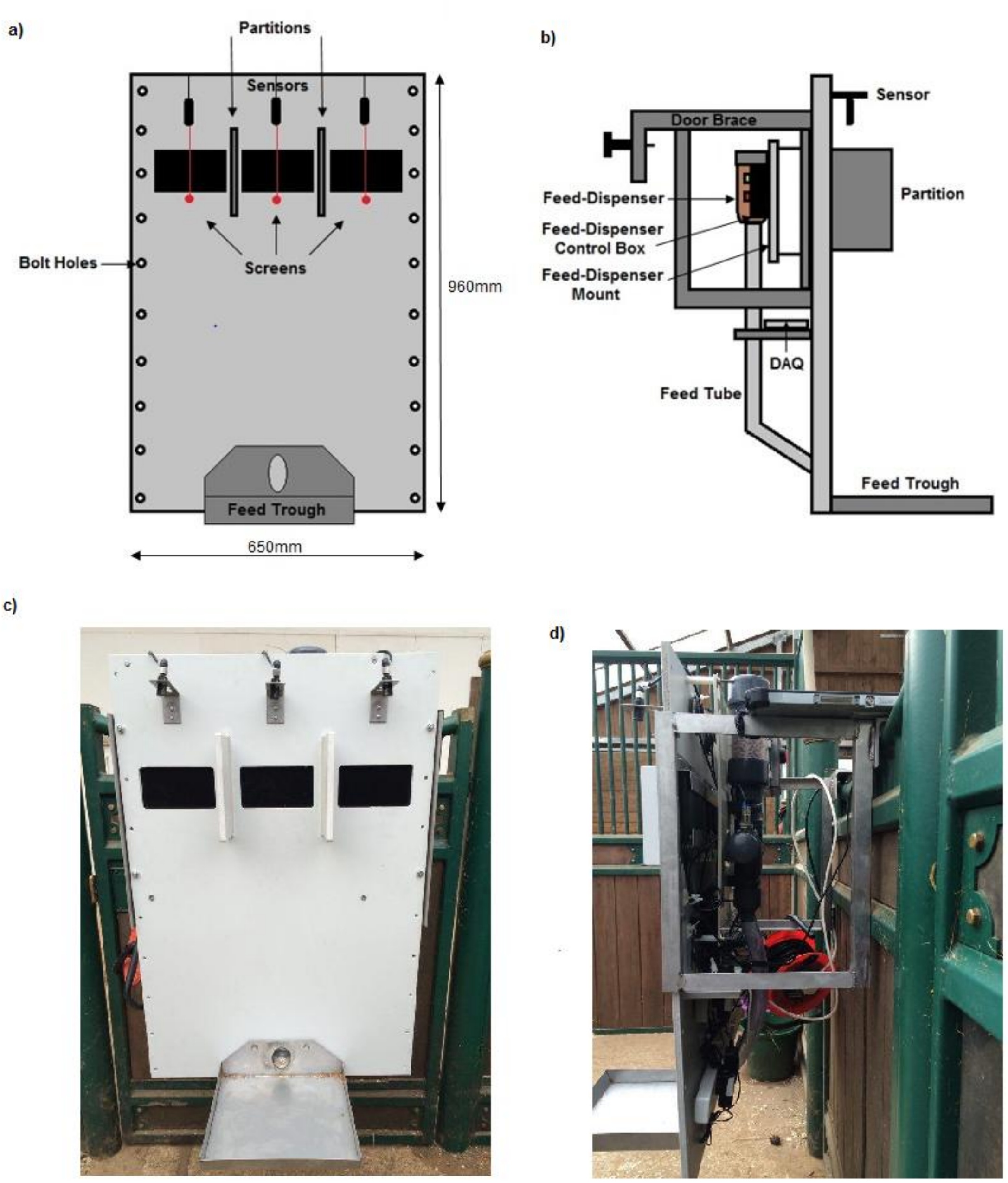

Figure 2. The set-up for the equine OS. a) and b) are the diagrammatic specification of the front and side of the OS respectively, whilst c) and d) demonstrate the front and side view of the OS as presented to the horse within the stable. The OS operator stands to the right, next to the door braces, ensuring no view of the screens.

Figure

Plate 1. Performing the 3-CSRTT. NB image for demonstration purposes only, under experimental conditions the human is located to the immediate right hand side of the OS as to be unaware of CS presentations.