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Review paper

Applied neurophysiology of the horse; implications for training, husbandry and welfare

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ABSTRACT

Understanding the neural circuits underlying equine behaviour has the potential to help optimise strategies of husbandry and training. This review discusses two areas of neurophysiological research in a range of species and relates this information to the horse. The first discussion focuses on mechanisms of learning and motivation and assesses how this information can be applied to improve the training of the horse. The second concerns the identification of the equine neurophysiological phenotype, through behavioural and genetic probes, as a way of improving strategies for optimal equine husbandry and training success. The review finishes by identifying directions for future research with an emphasis on how neurophysiological systems (and thus behaviour) can be modified through strategic husbandry. This review highlights how a neurophysioloigical understanding of horse behaviour can play an important role in attaining the primary objectives of equitation science as well as improving the welfare of the horse.

1. Introduction

Much is known about the behaviour of horses as a result of an increasing body of applied research. This greatly improves our ability to keep and train the horse in optimal ways. Understanding the neural circuits underlying equine behaviour has the potential to further optimise these strategies of husbandry and training. The aim of this review is to present general and equine-specific neurophysiological information within a translational framework, and by doing so, enhance the knowledge base of equitation science from a practical perspective. Two main areas of neurophysiological evidence will be discussed. The first relates to mechanisms of learning and motivation and thus the training of the horse. The second concerns the identification of the equine neurophysiological phenotype, through behavioural and genetic probes, as a way of improving strategies of equine husbandry and training. The review concludes with a discussion on areas of future research focusing on how neurophysiological systems can potentially be modulated, in a way that is both beneficial to horse (welfare) and rider (performance). In addition, the issue of performance versus welfare will be addressed throughout the review, with an emphasis on how added neurophysiological insight has the potential to help improve performance without diminishing welfare and vice versa. The review will start by outlining the key anatomical structures of the equine central nervous system that are relevant to behavioural output.

2. Equine central nervous system relevant to behavioural output

In order to behave appropriately within a shifting environment, the horse's brain must gather information from sensory receptors such as the retinae and epithelial somatosensory receptors, and convey these via the sensory branches of the peripheral nervous system (PNS) to the spine and then the brain. Following a process of integration, influenced by a combination of genomic, genetic and epigenetic processes as well as prior experience (learning), an appropriate behavioural response is issued to effectors via the motor pathways of the PNS. This section will focus on the primary brain structures involved in the integration process, with a focus on the basal ganglia, a group of structures that are pivotal in the ordering of behavioural output.

2.1. Basal ganglia

Fig. 1 presents the primary structures of the basal ganglia. These include the striatum, the subthalamic nucleus (STN), globus pallidus (GP; internal: GPi; external: GPe), substantia nigra (SN; pars

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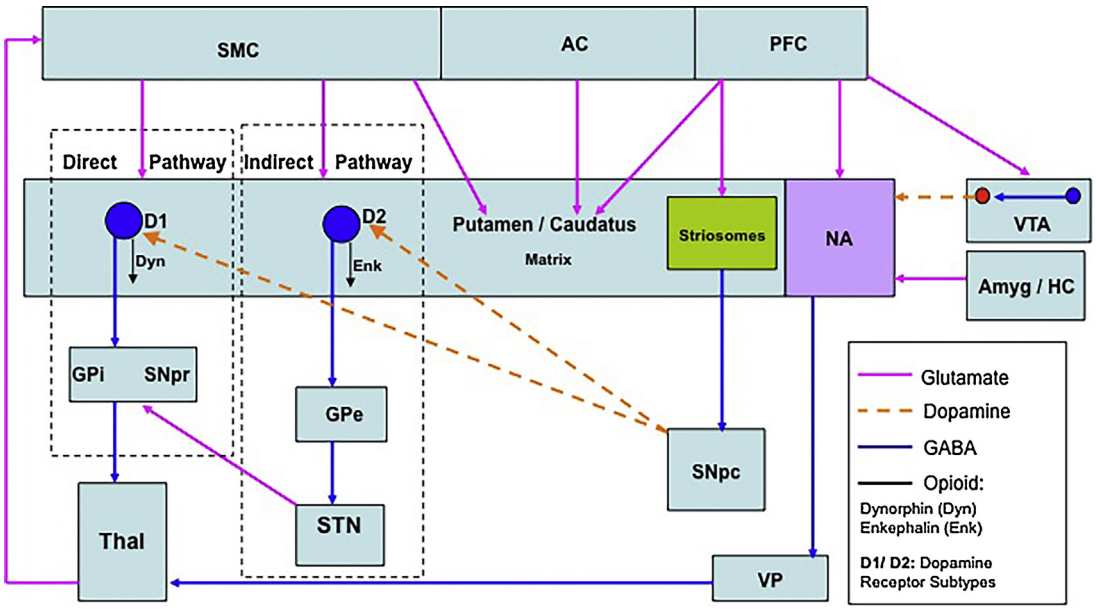

Fig. 1. Simplified schematic depicting primary brain structures and connectivity within basal ganglia-thalamo-cortical circuitry. Adapted from Haber et al. (2000); Lewis et al. (2006); Yin and Knowlton (2006) Graybiel (2008). Sensorimotor Cortex (SMC) Association Cortices (AC) Pre-Frontal Cortex (PFC) Caudatus (C) Putamen (P) Nucleus Accumbens (NA) Ventral Tegmental Area (VTA) Amygdala (Amyg) Hippocampus (HC)Globus Pallidus internal and external segments (GPi and GPe) Substantia Nigra pars compacta and pars reticulata (SNpc and SNpr) Sub Thalamic Nucleus (STN) Thalamus (Thal) Ventral pallidum (VP).

compacta: SNc; pars reticula: SNr) and ventral tegmental area (VTA). The striatum forms the main neural gateway to the basal ganglia and comprises the caudate, located dorsomedially (DMS), the putamen, located dorsolaterally (DLS), and the nucleus accumbens (NAc), located within the ventral aspect. The NAc can be further separated functionally and anatomically into the shell (NAcs) and core (NAcc) region. The striatum is made up of medium-spiny projection neurons (MSNs) and interneurons (cholinergic and GABAergic) (Voorn et al., 2004) that receive input (afferents) from the cortex, hippocampus, thalamus, amygdala, dorsal raphe nucleus, STN and from different mid-brain structures (SNc and VTA) (Haber, 2011; Wall et al., 2013). With regard to the cortex, there is a generalized but graded arrangement of cognitive control and reward areas (e.g. prefrontal cortex) sending projection neurons to the ventral striatum (NAc and ventral caudate), association areas (e.g. parietal cortex) innervating dorso-medial striatum and finally motor areas (e.g. motor cortex) innervating dorsolateral striatum (Haber, 2011; Choi et al., 2012) (Fig. 1).

Striatal MSNs have a 'many to one' arrangement such that only specific combinations of dendritic activity, which originate from a range of cortical and sub-cortical structures, may elicit an MSN action potential (Mink, 1996; Wickens and Arbuthnott, 2010). Research to date suggests that cortical information is transferred via the basal ganglia to the thalamus and back to the cortex through three different pathways; the direct and indirect pathway through the striatum and the hyperdirect pathway through the STN (Maurice et al., 1999; Gerfen and Bolam, 2010; Haynes and Haber, 2013).

The direct pathway consists of a direct inhibitory GABAergic projection from the striatum to the SNr/GPi which also projects GABAergic neurons onto the thalamus. This double inhibitory system means that cortical input into the striatum has the potential to disinhibit the thalamus allowing a return of information back to the cortex (Gerfen and Bolam, 2010) and an overall excitatory effect. Cells of the direct pathway are rich in dopamine receptors of the D1 family (comprising D1 and D5 sub-groups), which receive dopamine (dopamine) influx from the SNpc, (discussed in the following section) (Aosaki et al., 1998; Stolzenberg et al., 2010). By contrast, the net effect of indirect pathway activity is that of cortical inhibition (Presti and Lewis, 2005). This is achieved through inhibitory GABAergic projections from the striatum to the GPe which then projects inhibitory GABAergic neurons onto both the SNr/GPi and STN. The inhibitory neurons onto SNr/GPi form a triple inhibition system such that cortical initiation of the indirect pathway results in increased inhibition of the thalamic output and thus attenuated return of cortical information. MSN's of the indirect pathway express dopamine receptors of the D2 family (comprising D2, D3 and D4 subtypes), which are negatively coupled to adenyl cyclase stimulation (McPherson and Marshall, 2000). Activation of D2 populations by SNpc dopamine projections, effectively dampens the inhibitory drive associated with indirect MSN activity (Nicola et al., 2000), thereby disinhibiting the thalamo-cortical motor projection (discussed further in the following section)

The STN has historically been included as part of the indirect pathway but is now discussed as the hyper-direct pathway (Haynes et al., 2004). Primary STN efferents are glutamatergic and thus excitatory, targeting both pallidal structures of the direct and indirect pathway (SNr/Gpi and GPe respectively) (Charpier et al., 2010). Theoretically the STN has the opportunity to increase activation of the both the direct and indirect pathway but it is also modulated by the GABAergic feedback loop from GPe back to STN (Charpier et al., 2010). This complex relationship of the STN in modulating and being modulated by other basal ganglia structures results in a signature triphasic response of the SNr (the primary output structure to the thalamus); firstly it shows early excitation as a result of hyperdirect pathway activation via the STN, this is followed by inhibition as a result of the GABAergic direct pathway activation, and finally it shows late excitation as a result of the activation of the indirect pathway (Nakanishi et al., 1988; Maurice et al., 1999; Magill et al., 2004).

2.2. Dopaminergic modulation of basal ganglia via mid-brain structures (SNc and VTA)

Dopamine is integral to striatal synaptic activity, with striatal excitatory post-synaptic potentials (EPSPs) largely modulated by

mid-brain dopamine innervation (Gerdeman et al., 2003; Di Filippo et al., 2009). The effect of dopamine transmitter release on the striatum, however, is dependent on the type of MSN within the striatum. For MSNs that express D1 dopamine receptors, muscarinic M4 receptors, dynorphin (κ-opioid receptor) and substance P, dopamine has an excitatory effect, stimulating the direct pathway (Kreitzer, 2009). For MSNs that contain D2 dopamine receptors and adenosine A2a receptors, and express enkephalin (delta opioid receptor), dopamine has an inhibitory effect, stimulating the indirect pathway (Kreitzer, 2009). Dopamine neurons from the SNc (nigrostriatal pathway) and VTA (mesoaccumbens pathway) directly target different striatal structures with the SNc modulating MSNs within the dorsal striatum (caudate and putamen) whereas the VTA modulates the ventral striatum (nucleus accumbens) (Kalivas et al., 1983). Neurons along the nigrostrital and mesoaccumbens that fire continuously at a low firing rate are referred to as 'tonic' whereas sudden increases in firing rate as a result of environmental effects are referred to as 'phasic' dopamine release (Surmeier et al., 2007).

2.3. Reciprocal and non-reciprocal loops from the striatum to mid-brain structures

A major feature of striatal to mid-brain connectivity is the mechanism of reciprocal feedback combined with non-reciprocal feed-forward. This allows regions of the striatum to modulate their own dopamine afferents (reciprocal feedback) but also to affect mid-brain dopamine modulation of other regions of the striatum (non-reciprocal feed-forward) (Haber et al., 2000). This arrangement is such that dopamine innervation of the NAc, as well as resulting in feedback to this same region, also has a feed-forward effect on the caudate (DMS) portion of the striatum. What is critical to this mechanism is that feedback is through a direct mechanism, which causes inhibition, but feed-forward is via indirect GABA inter-neurons which has an overall excitatory effect. In a similar fashion, DMS negatively feedbacks on itself but positively feeds to the DLS (Haber et al., 2000).

3. Learning and motivation

Operant learning theory is partitioned into positive reinforcement, negative reinforcement and punishment. Reinforcement is the strengthening of a behavioural response to a discriminative stimulus [cue] either through the introduction of an appetitive stimulus [positive reinforcement] or the removal of an aversive stimulus [negative reinforcement] as a consequence of the animal's response. Conversely, punishment is the weakening of a behavioural response to a discriminative stimulus by the introduction of an aversive stimulus [positive punishment] or the removal of an appetitive stimulus [negative punishment] again as a consequence of the animal's response. Within equitation training methods, negative reinforcement methods predominate, although there is increasingly a greater integration of positive reinforcement techniques within training programmes (Warren-Smith and McGreevy, 2007; Warren-Smith and McGreevy, 2008; Hockenhull and Creighton, 2010). Within reinforcement learning there are also three sequential phases of the learning process, acquisition, action-outcome and habit formation (Dickinson, 1985). For the purposes of understanding how the basal ganglia orchestrates these learning processes, the mechanisms underpinning learning through positive reinforcement will be discussed first, and then extended to describe the three aforementioned phases of the generalized learning process. This will provide a basis for discussion of the mechanisms underpinning both negative reinforcement and punishment.

3.1. Neural mechanisms underpinning positive reinforcement

Through a process of associative learning, objects, environments and events become predictive of the arrival of other biologically relevant stimuli where the attainment (positive) or removal (negative) of those stimuli are linked to specific behavioural sequences (operant response) (Yin et al., 2008). An operant response to attain a reward is referred to as positive reinforcement whereas an operant response in order to remove a negative stimulus (escape) or to avoid the arrival of a negative stimulus (avoidance) is referred to as negative reinforcement. Once learnt, subsequent presentation of cues grab attention and elicits motivation towards appetitive/evasive behaviours to re-attain the same goal (Mackintosh, 1975). Fundamental to these learning processes are the biological phenomena of long-term potentiation (LTP) and long-term depression (LTD) that strengthen or weaken the link between sensory input and motor output. Cortical neurons, containing both sensory information about the environment as well as a continual log of what actions the animal has been performing (motor efferent copy), terminate at medium spiny neurons within the striatum (Levesque et al., 1996; Lei et al., 2004; Redgrave et al., 2008). If the action leads to the arrival of a reward, then the neural circuitry between these two pieces of information (sensory and motor) is strengthened. This process is achieved through LTP as a result of phasic dopamine released and activation of D1 dopamine receptors on MSNs within the striatum (both ventral and dorsal) (Surmeier et al., 2007; Lovinger, 2010). Conversely, if the sensory information no longer predicts the arrival of salient stimuli, then the reverse process of LTD, through dips in tonic dopamine release, diminishes the link with the motor output (Surmeier et al., 2007; Shen et al., 2008). The latter describes the process of extinction when the lack of reward presentation eventually produces no response from the animal.

What is important to note is that changes in dopamine release are determined by the difference between what the animal expects as a reward and what is actually delivered. This differential value is referred to as the 'reward prediction error' (Schultz et al., 1997). The dopamine response is greatest the first time the reward is presented (because it is completely unexpected), but then wanes with multiple presentations and is only re-kindled when the reward value is increased or if temporal unpredictability is re-inserted (Bayer and Glimcher, 2005). Conversely, if reward is expected but not presented, this results in a reduction in tonic dopamine release. For example, in water deprived primates, bursts of activity in the dopaminergic VTA neurons were observed when unexpected juice was dispensed, whereas suppressions in tonic firing rate were recorded when expected rewards were withheld (Mirenowicz and Schultz, 1994; Schultz, 2002).

Post-acquisition, once an operant response has been learnt, phasic release of dopamine at the point of the striatum moves temporally from the point of reinforcer delivery to the point of cue/conditioned stimulus (CS) presentation (Ljungberg et al., 1992; Schultz, 1998) (Fig. 2). It is considered that this (1) acts to allow only certain permutations of cortical afferents to pass through the striatum (i.e. those associated with salient stimuli) and (2) codes the expected outcome of the action (Samejima et al., 2005; Morris et al., 2006). In this sense, the CS dopamine signal appears to have a gating function for specific cortical glutamatergic signals arriving at the striatum (Frank et al., 2001) that, through an system of competition, provide the neural architecture for an action selection process (Prescott et al., 2006). The CS dopamine signal post-acquistion also arrives at the ventral striatum via the mesoaccumbens (VTA-NAc) dopamine pathway (Schultz, 1997). Yin et al. (2008) postulated that phasic dopamine activation of MSNs in the NAc acts as an amplifier of actions that have already been selected. This determines the vigour by the which the behaviour is performed, often referred

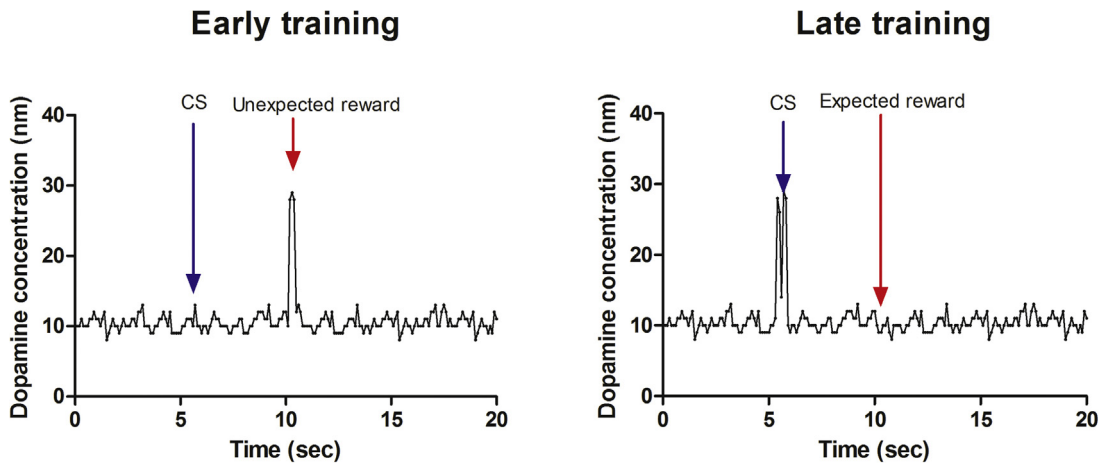

Fig. 2. Changes in phasic dopamine concentration (nM) within the striatum as a result of unexpected reward during early training and the presentation of a conditioned stimulus during late training. Note the lack of dopamine response during an expected reward (Schultz, 1986; Ljungberg et al., 1992).

to as motivation (Yin et al., 2008), and is the considered mechanism whereby motivational properties are conferred to conditioned stimuli (Yin et al., 2008). The strength of this signal is determined by the valence (positive or negative) of the unconditioned stimulus to which the CS is linked. Anatomically, it is the previously described non-reciprocal feed-forward connections via the mid-brain that allow the ventral striatum to modulate the gating processes of the dorsal striatum and thereby have this amplifying effect (Haber et al., 2000).

3.2. The three phases of the learning process: acquisition, action-outcome and habit formation

The generalised arrangement of cortico-striatal loops and the heterogeniety of the striatum, as previously described, is considered to relate functionally to the three primary stages of learning. The initial 'stamping' in of behaviour, that makes the link between logged sensory and motor information as described in the previous section, is considered to occur at the level of both the ventral (nucleus accumbens) and dorsomedial (caudate) striatum (Yin and Knowlton, 2006; Redgrave et al., 2008). Activation of these cortico-striatal loops continue through the acquisition process but, once the behaviour has become learned, loops through the dorsomedial striatum start to predominate. This reflects a shift towards action-outcome learning where the animal, having learned the behaviour, maintains a process of closely monitoring the consequences of its actions in the context of expected outcome. The final stage of learning is habit formation, which occurs through a process of over-training where the animal has repeatedly received the same outcome for its actions. Neurophysiologically, this is associated with a dorso-lateral shift of cortico-striatal loops away from the caudate (DMS) towards the putamen (DLS). During action-outcome learning, both devaluation of the outcome or degradation of the contingency between the response and outcome, reduce the level of responding. During habit formation, responding is less affected by either of these manipulations as a result of reduced monitoring of action outcome (Haber and Calzavara, 2009).

The fact that over-trained behaviours are more resistant to changes in contingency (from both a negative and positive reinforcement perspective) strongly suggests that there is less dopaminergic influence over the learning system during habit formation. Neurophysiological studies indicate that this is indeed the case (Wickens et al., 2007; Graybiel, 2008). However, all behaviours, no matter how ingrained they are through overtraining, will eventually be extinguished through lack of reinforcement and the dopamine-mediated physiological process of LTD (as previously described).

Habit formation is critical during horse training because it can both facilitate and impede the learning process. For example, the ability to maintain a specific set of behavioural sequences without having to maintain dopaminergic-based reinforcement means, in practical terms, that aversive pressure can be removed from training whilst still maintaining the desired level of response. Similarly, habit formation can also be advantageous during positive reinforcement training. For example, one of the potential problems of positive reinforcement training programmes is the limit on work that an animal will perform in order to attain a non-essential resource (e.g. a reinforced sound such as a 'click' from a handheld device followed by a small amount of cereal-based concentrate) (Dawkins, 1991). Habit formation potentially circumnavigates this problem by allowing each incremental step of training (where the complexity and physical demand of the operant response is increasing) to be trained to a point where the animal is no longer monitoring the outcome (reward) of its action. To explain this in neurophysiological terms, habit formation alleviates the need for dopaminergic reinforcement does not need to be cumulatively applied at each incremental step of the task. This theoretically allows work load to be increased without requiring a concurrent increase in the quantity of reward presented to the animal.

The potential disadvantage of habit formation, however, is that in its extreme form it can reduce behavioural flexibility. The inability to shift from one learnt behaviour to another, often referred to as perseverance (Garner et al., 2006), will mean that the animal cannot progress through the normal stages of successive training goals, as the complexity of the desired behavioural response is built up. Factors that can produce abnormally high perseverance and habit formation are discussed in Section 4.

3.3. Neural mechanism underpinning negative reinforcement and punishment

Training through negative reinforcement can inadvertently lead to the attenuation of other behavioural sequences through the physiological consequences of punishment. To give an example of this, a cue in the form of light non-aversive leg pressure to move the horse forward, may through insufficient training, not lead to the desired behaviour. A typical response to this by the rider is to increase leg pressure. This switches the stimulus from being a cue to being an aversive event which, has the potential to act both as a negative reinforcer and as a punisher. A negative

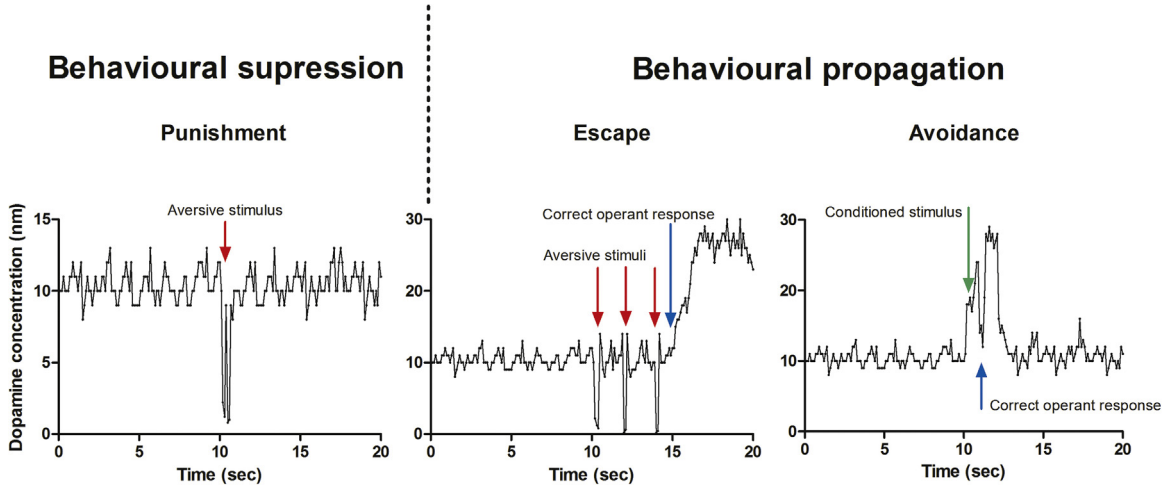

Fig. 3. Changes in tonic and phasic dopamine concentration (nM) within the striatum as a result of presentation of aversive stimuli in the context of punishment, escape and avoidance (Oleson et al., 2012; Wenzel et al., 2015).

reinforcer to produce the desired behaviour (in this example move forward) or as a punisher of a concurrent behaviour that the animal is currently engaged (for example, collected walk). The timing of increased and decreased dopamine release within the striatum during the different stages of this process potentially gives some insight to the processes involved. Although aversion-based learning processes are complex in terms of the types of neurons that are firing (and the different regions of the striatum involved (Wenzel et al., 2015)), recent studies are starting to provide some clarification of the underlying mechanisms involved. For example, the application of unexpected aversive stimuli appears to consistently lead to the cessation or a reduction in the tonic firing rate of the mesoaccumbens dopamine pathway (VTA to NAc) (Matsumoto and Hikosaka, 2009; Cohen et al., 2012). This diminishes the strength of the neural link between the stimulus (whatever was occurring at that moment) and any behavioural sequence that was logged prior to, or during, the application of the aversive stimulus (Tobler et al., 2003). This process is similar to what occurs neurophysiologically when a reward is expected but not delivered (extinction). However, if that same aversive event is paired with a CS, the presentation of the CS, as an indicator of the aversive event to come, will lead to an increase in dopaminergic activity within the ventral striatum as a way of invigorating active avoidance behaviour in the animal. Oleson et al. (2012) using a signalled operant shock avoidance task, trained animals to a conditioned stimulus (light) that indicated the arrival of an electric foot shock after a 2 s interval. A lever press during this 2 s interval resulted in the avoidance (non-presentation) of the foot shock. If the animal did not respond, recurring foot shocks were applied until the animal did respond with the lever press. Behaviourally, during the early stages of training, animals reverted to species-specific defence behaviour, which in the case of rodents was freezing behaviour, in response to both the application of the aversive stimulus and its conditioned cue. Physiologically, the early stage of training was associated with a drop in tonic dopamine release within the ventral striatum during both events. As training continued, the animals learned to stop the electric shock through lever pressing, and this was associated with a surge in dopamine release within the ventral striatum. Finally, the animal learned to avoid the electric shock in advance by pressing the lever in response to the predicting cue. The transition to this final stage of learning was associated with a significant increase in dopamine release within the NAc at the point of cue presentation. These different effects of aversive stimuli when presented in different contexts are summarised in Fig. 3. This study is extremely relevant to equitation and horse training as it closely mimics the typical sequence of events that occur during most training programmes, and thus it gives potential insight to the neurophysiological processes involved. For example, the question asked at the beginning of this section was how the animal differentiates between negative reinforcement (to promote behaviour) and punishment (to suppress behaviour). At the level of dopamine physiology, it appears that the initial application of an aversive stimulus has the potential to suppress specific behaviours being performed at the point of aversive stimulus presentation. In rodents, this manifests as behavioural suppression (freezing), whereas for the horse the primary species-specific defence response is flight. In this context, punishment in the horse has the paradoxical effect of simultaneously elevating general behaviours associated with flight, whilst at the same time suppressing specific behaviours performed at the time of the aversive stimulus presentation. The critical transition from punishment to negative reinforcement appears to occur as a result of repeated application of the aversive stimulus. This propagates trial and error behaviour as the animal attempts to escape the stimulus. Successful escape elicits dopamine release within the ventral striatum and this is the crucial point at which learning occurs during negative reinforcement (Wenzel et al., 2015). If this process occurs in the context of a CS, dopamine release shifts temporally backwards to that point (as it does during positive reinforcement) to elicit and invigorate avoidance behaviour that prevents the onset of the aversive stimulus (Fig. 3).

Further research is still required to elucidate the physiological consequences of different timings and duration of aversive stimuli. As stated at the outset of this discussion, switching between negative reinforcement and punishment can easily occur during the riding and training process. For example, what is happening neurophysiologically when an operant response learned through negative reinforcement, now elicits the presentation of an aversive stimulus? In the Oleson et al. (2012) study, this would equate to, the lever press now eliciting the electric shock rather than preventing it. This type of switching from avoidance to punishment has previously been shown to dramaticlly suppress dopamine physiology and the general level of behaviour that the animal performs (Hikosaka, 2010; Bressel and McNally, 2014). Although not well defined in the context of equine behaviour, the term ‘frozen watchfulness’ (which describes emotional maltreatment in humans (Walsh, 1990)) has been used to describe horses that are hyper-vigilant but unresponsive to environmental stimuli. It may be that perpetual shifts from negative reinforcement to

punishment is the basis of this behavioural condition in horses. Overall, the studies discussed in this section highlight some of the risks of training under negative reinforcement systems and emphasise the critical importance of timing, where, when poorly implemented, can lead to unexpected aversive stimuli becoming punishers that suppress rather than promote behaviour.

4. Predictive markers of the neurophysiological and behavioural phenotype

The term endophenotype describes the categorisation of an animal's behavioural traits in relation to its genetic profile (John and Lewis, 1965). It describes the predisposition to express specific categories of normal and abnormal behaviour as a result of the genotype-environment interaction. For example, the trait of 'mood instability' is considered to be an endophenotype for attention deficit hyperactivity disorder (ADHD) in relation to specific polymorphic markers within the dopamine transporter gene DAT1 (Jeong et al., 2015). In addition, the inability to differentiate different reward values of stimuli during a reward learning task is considered to be an endophenotype for depression (see Goldstein and Klein, 2014, for review).

The concept of endophenotype is relatively unexplored in the horse but has huge potential to be developed given the behavioural profiling that can be easily undertaken and the affordable genetic testing that is now available. This would allow the behavioural effects of training and husbandry to be clearly defined in the context of genetic predisposition. For example, the consequence of different weaning strategies have been shown to have a large impact on the behaviour of some animals (Waters et al., 2002). The use of behavioural and genetic probes could be used to identify susceptibility to this and other early-life event stressors. Furthermore, upregulation of dopamine systems can occur through consistent exposure to highly palatable substrates (Colantuoni et al., 2001), and this can have extensive ramifications on behaviour, as discussed in the previous sections. Again, the animal's predisposition to changes in neurophysiology and behaviour as a result of nutritional factors could be identified through behavioural and genetic markers.

Table 1 presents a summary of current and potential behavioural/cognitive tests that can be applied to the horse in order to extract information about neurophysiological status and/or predisposition of the animal. These tests have been selected because the information that they provide is of potential relevance to the practical training and husbandry of the horse. In the following sections, we outline three of these tests and discuss their relevance from an applied perspective. This is followed by a review of current genetic markers that also have the potential to identify the equine endophenotype in a way that might be practically useful.

4.1. Spontaneous blink rate

As discussed, basal ganglia function and thus behavioural output, is profoundly influenced by dopamine neurons originating in the midbrain. Therefore, any behavioural markers of dopamine transmission will provide valuable insights into the neural basis of equine behaviour and cognition. Spontaneous Blink Rate (SBR) measurement is showing considerable promise in this regard. SBR is described as bilateral paroxysmal brief repetitive eye closures that occur continuously and in the absence of obvious external stimuli, and is not affected by external stimuli such as light, heat or humidity (Karson, 1983).

Administration of dopamine agonists and antagonists has been used to validate the SBR as an indirect measure of dopamine tone within the CNS. For example, Karson (1983) administered 0.36 mg/kg of the dopamine agonist apomorphine to four Rhesus Monkeys and observed a four-fold increase in SBR which could then be abolished following injection of the dopamine antagonist sulpiride. Therefore, elevated dopamine within the CNS increases spontaneous blinking whereas dopamine suppression has the opposite effect. Additional evidence for the use of SBR comes from human neurodegenerative disorders whereby depleted dopamine due to Parkinson's disease will also lead to lowered SBR (Karson 1983). Similarly, disorders resulting in dopamine facilitation (i.e schizophrenia) produce significant elevations in SBR (Mackert et al., 1991). In relation to horses, neuro-degeneration of dopamine neurons terminating in the hypothalamus as a result of Cushings syndrome, is associated with significantly depressed SBR (Stephenson et al., 2014). Interestingly, Roberts et al. (2015) recorded lowered SBR in horses performing oral stereotypic behaviour (crib-biting). Post-mortem studies of crib-biting horses have demonstrated widespread alterations to dopamine function at the level of the striatum (McBride and Hemmings, 2005), with evidence of significantly lowered dopamine transmission into the caudate, in tandem with up-regulated dopamine activity in the NAc. The SBR depression recorded by Roberts et al. (2015) would therefore appear to reflect the decreased activity in the caudate reported by McBride and Hemmings (2005) rather than the facilitation into the nucleus accumbens. In support of this notion, neural control of SBR depends on dopamine transmission from the substantia nigra (Kaminer et al., 2011), a midbrain region supplying dopamine exclusively to the caudate and putamen. SBR analysis in horses, therefore, has the potential to identify behavioural phenotypes in relation to stereotypy development.

High levels of SBR in horses have also associated with an inflexible approach to problem solving in a discrimination reversal task (Hemmings, 2012). These findings are supported by rodent work that has demonstrated that elevated levels of dopamine results in accelerated habit formation in rats (Nelson and Killcross, 2006). As previously discussed in section 3.2, habit formation can be both advantageous and disadvantageous from a training perspective. Using SBR to identify animals with elevated dopamine, and thus predisposition to accelerated habitual responding, may lead to more a targeted application of specific training approaches.

4.2. Behavioural switching frequency

The rate at which an animal transitions between behaviours (e.g. eating to standing alert to walking to eating) is referred to as Behaviour Switching Frequency (BSF). Used in tandem with measures of SBR, BSF measurements can provide additional insight into dopamine function at the level of the striatum. The evidence base in support of this notion is similar to that already covered for SBR. To summarise, dopamine agonist administration induces elevations in BSF which may be mediated by dorsal and/or ventral elements of the striatum (Robbins and Sahaikian, 1983; Cabib, 1993; Garner, 2006). Interestingly in a study in stereotypy horses (Roberts et al., 2015) BSF was significantly elevated in both crib-biters and weavers compared to non-stereotypy performing controls, but only the crib-biting group displayed significant differences in the levels of SBR. This suggests neurophysiological differences between oral and locomotor stereotypy animals but also that two behavioural tests are measuring different neurophysiological aspects of basal ganglia function. Given the importance of striatal function to a spectrum of equine behavioural responses, as discussed in the previous sections, BSF and SBR are potential markers of both current and future behavioural phenotypes, for example, stress susceptibility, habit formation, flexible learning and stereotypy predisposition. Future research using these markers within longitudinal studies will allow the neurophysiological state of the animal to be tracked

Table 1
A summary of cognitive and behavioural tests to profile the equine endophenotype from an applied perspective.

Test	Description	Psychological metric	Previously tested in horses	Application to equine training and welfare
Progressive ratio	The number of correct operant responses for a reward is increased progressively. The point at which the animal stops responding is referred to as the break point (Roberts et al., 1989)	Measures motivation and apathy	Yes (Houpt, 2012)	Potential marker of hyper-motivation and stereotypy predisposition? Marker of reward sensitivity
Extra-intra-dimensional shift/discrimination-reversal	Two-choice discrimination and reversal of visual object based on different rules e.g. shape and colour (Dias et al., 1996)	Measures flexibility of learning and attention	Yes (Sappington et al., 1997; Martin et al., 2006)	Marker for flexible learning critical for complex tasks and successive approximation
Extinction Learning	Animal is taught an operant response for food reward. This is followed by removal of the positive reinforcer. Latency/trials to response cessation is recorded.	Measures perseverance/habitual responding	Yes (Hemmings et al., 2007)	Identifies animals which may be prone to habitual response patterns during training
5 choice serial reaction time test	Operant movement towards one of five briefly (e.g. 0.5s) lighted areas with errors of movement recorded during the inter-trial interval (e.g. 5s) (Weed et al., 1999)	Measures attention and impulsivity	No	Marker for impulsivity and poor attention which will affect training rate. May also be a potential marker for stereotypy predisposition.
Concurrent schedules/concurrent chain schedules	Choosing between two concurrently presented stimuli, each of which is associated with a different reward value (Grace et al., 1998)	Measures motivation and impulsivity.	Yes, (Parker et al., 2008)	Potential marker for impulsive choice/lack of self-control which will affect training rate. May also be a potential marker for stereotypy
Place-response learning	Animal is trained continuously to approach a goal in the same arm of a T-maze. In a probe trial, the animal enters the maze from the opposite arm to training (Tolman and Gleitman, 1949)	Measures habit formation	Yes (Parker et al., 2009)	Potential marker for stereotypy. Potential marker for behavioural inflexibility and habit formation which will have implications for learning ability
Pre-pulse inhibition	Exposure to a weak 'pre'pulse attenuates the reaction to a stronger stimulus (pulse)	This is a measure of the startle reflex, and is known to be disrupted in patients with neurological conditions and some neuropsychiatric disorders	No	Potential marker for poor impulse control and poor executive function. This will have implications for learning ability
Judgement bias	Animals are trained on two conditioned stimuli (positive and negative) and then presented with an ambiguous conditioned stimulus (Harding et al., 2004)(Harding et al., 2004)	The response to the ambiguous conditioned stimulus is a potential measure of optimism/pessimism and an inferred measure of emotional state.	Yes (Freymond et al., 2014)	A potential welfare indicator of training techniques and husbandry environments. May also be a useful probe for 'mood' in relation to performance (McBride and Mills, 2012)
Spontaneous blink rate	Rate of bilateral brief repetitive eye closures that occur continuously and in the absence of obvious external stimuli, and is not affected by external stimuli such as light, heat or humidity. (Karson, 1983).	Habit formation and impulsivity	Yes (Roberts et al., 2015)	As an indirect measure of dopaminergic tone, this may be a useful marker for stereotypy predisposition, accelerated habit formation and/or impulsivity.
Behavioural switching frequency	Using a defined species-specific ethogram, this test monitors the rate of switching from one behaviour to another (Garner, 2006)	Habit formation and impulsivity	Yes (Roberts et al., 2015)	As an indirect measure of dopaminergic tone, this may be a useful marker for stereotypy predisposition, accelerated habit formation and/or impulsivity.
Pavlovian Instrumental transfer	A classically conditioned association is shaped to become contingent upon an operant response.	Rate of transfer from autonomic to voluntary mediation of behavioural output	Yes (Lansade et al., 2013)	Relevance to learning mechanisms underlying secondary reinforcement (e.g. clicker training)

throughout its lift time in relation to key husbandry and management events.

4.3. Extinction learning paradigms and perseverance

To date, extinction learning paradigms have been employed to advance our understanding of neural dysfunction in animals performing stereotypic behaviour (Garner and Mason, 2002) or in experimental scenarios that investigate substance addiction (Nelson and Killcross 2006). Although there is significant protocol variation between research groups, animals are generally taught a simple operant task (usually for a food/drug reward) followed by cessation of the reward to break down the action-outcome contingency. Response rate and or trials to extinction (cessation of operant responding) are then recorded as a measure of 'perseverance' (Hemmings et al., 2007).

In relation to stereotypic behaviour, bank voles performing high levels of oral stereotypy, were less likely to suppress a previously learned response in a spatial extinction paradigm (Garner and Mason 2002), a behavioural symptom that has been strongly linked to basal ganglia dysfunction in human patients with autism (Turner, 1997), and schizophrenia (Frith and Done, 1983). More recently,

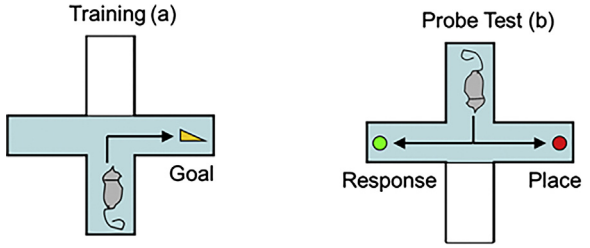

Fig. 4. Place response task incorporating the cross maze. During the training phase (a) rats are trained to enter the south entrance followed by a right turn to retrieve a food reward. During a probe test (b) the entrance is switched to the northern arm and food is removed. A left turn in the direction of the food reward during training indicates an A–O driven 'place strategy', a right turn an inflexible 'response strategy' (re-drawn from Yin and Knowlton, 2006).

similar impairments in extinction learning have also been recorded in stereotypy-performing blue tits (*Cyanistes caeruleus*), marsh tits (*Poecile palustris*) (Garner et al., 2003) and several species of bear (Vickery and Mason, 2005), leading to the conclusion that stereotypy performance in captive animals is associated with altered basal ganglia output. In relation to equine stereotypy, Hemmings et al. (2007) subjected 10 crib-biting horses and 10 stereotypy free controls to a standard extinction learning paradigm. Perseverance scores for the cribbing group were significantly higher compared to the control horses giving further support to the link between perseverance, basal ganglia dysfunction and stereotypy performance.

Measures of perseverance also have the ability to predict predisposition to habit formation which, as discussed in Section 3.2, is a critical factor in determining the animal's aptitude for adaptability during learning and training. Tests that can measure learning adaptability, or predisposition to adaptability later on in the animal's life, may therefore be advantageous. Extinction learning tasks, however, generally require the use of specialized operant devices to deliver conditioned stimuli and food rewards, in such a manner that body cues from the experimenter are eliminated. This equipment is generally not available commercially so considerable expertise is required during the design and construction phases. Consequently, probes for extinction learning will almost certainly fall beyond the means of the horse owner/trainer. This is unfortunate, as tests for habitual responding would be of use in order to prevent over-training, or as mentioned previously to identify animals predisposed to stereotypy development. A solution to this problem is to use simple maze tasks that can be set up in most equestrian establishments. These tests do not require a response shaping phase and thus also reduce the need for training expertise. At the time of writing, the use of maze tests to reveal alterations in equine brain function had not progressed beyond the Tolmans Place/Response task. Developed originally by Edward Tolman, rats were trained to enter from the South and make a right turn for a food reward (see Fig. 4a), this process is then repeated between 8 and 70 times (Tolman and Gleitman, 1949). A probe test is then conducted to establish whether this well-practiced routine has progressed towards habit formation. Conducted only once, the probe test starts with the animal's introduction into the North arm (see Fig. 4b). An automatic right turn indicates progression into habit, also referred to as a 'Response' strategy. A left turn towards the pre-rewarded Easterly arm signals a 'Place' outcome. The latter indicates that the animal is still deploying a more flexible non-habitual approach to the task. From a neural systems standpoint, activity of the caudate (dorsomedial striatum) is associated with flexible place learning, whilst the putamen (dorsolateral striatum) appears to mediate the habitual response (Yin and Knowlton, 2004). Indeed, reversible and irreversible lesions of the dorsomedial striatum forces habitual responding, even in the absence of overtraining (Yin et al., 2005). Conversely, dorsolateral lesions prevent the formation of habit, despite multiple repetition (Yin et al., 2004). A less pronounced analogue of dorsomedial inactivation is observable in the crib-biting horse, whereby post mortem evidence suggests significantly decreased dopamine neurotransmission into the caudate nucleus (McBride and Hemmings, 2005). On this basis (Parker et al., 2009) hypothesized that crib-biting horses would align with the rodent studies (i.e Yin et al., 2005) and display a significant bias towards habitual responding, which indeed they did. These results suggest that maze testing is effective in identifying differential functioning of the dorsal striatum particularly in relation to accelerated habit formation. The use of maze tests may, therefore, be a simple and cheap, yet effective, marker for identifying learning and training adaptability in the horse.

4.4. Genetic predictors of the behavioural phenotype

The current glut of unwanted horses in the UK, Ireland, Australia and United States is partly due to indiscriminate breeding and a lack of targeted selection policy. However, with the sequencing of the equine genome in 2007, comes the opportunity to inform breeding strategy with genomic marker assisted selection. There now exists a handful of commercial enterprises devoted to the prediction of racing success based on markers of physicality such as muscle mass (Hill et al., 2010) and mitochondrial output (Harrison and Turrion-Gomez, 2006). Along with the physical components of athleticism, a suitably trainable and manageable temperament is required for fruitful propagation of the horse human bond. From a genetic standpoint, anecdotal reports from experienced horse handlers often apportion a heritable component to aspects of temperament, with certain stallions gaining notoriety based on unmanageable behavioural traits of their progeny. It could be argued that selection for temperament (albeit unwitting) has been ongoing from the point of domestication, whereby only those animals with suitably passive behavioural tendencies would lend themselves to initial recruitment and subsequent coping within early management systems (Hemmings and Hale, 2013). The longevity of any equine athlete will depend largely upon its ability to cope with restricted locomotion, social isolation as well as ad libitum feeding behaviour. Therefore, the aim of this section is to review work on equine behavioural genetics with a focus on coping style and traits with relevance to training and performance, such that future research efforts may be adequately informed.

4.5. Dopamine receptor genes in relation to temperament

Human studies have revealed a significant association between polymorphisms of the dopamine Receptor D4 (DRD4) locus, with traits such as novelty seeking and substance abuse (Lusher et al., 2001). This finding was the basis of a study by Momozawa et al. (2005) using a population of Japanese Thoroughbreds. In the 136 horses analysed, an A-G substitution was significantly associated with two temperament traits (curiosity and vigilance). Animals without the A allele had higher curiosity and lower vigilance scores. Both of these behavioural tendencies could be argued as important traits in the training environment. Hyper-vigilance in particular, has been linked to inappropriate initiation of the flight response, in circumstances that might cause danger to human handlers, or undue energy expenditure within the stable. Increased curiosity, rather than neophobia, could be argued to be a desirable trait, when introducing naïve animals to novel environments, maybe in the competition setting. Selection based on the A allele (either in parents or cohorts of young animals) might therefore lead to positive outcomes in competition and aspects of well-being linked to confinement.

Other human research has also linked DRD4 polymorphisms to impulse control disorders, which are important as increased

impulsivity is proposed to be a precursor of psychiatric illness, with behavioural inhibition data supporting the role of dopamine within impulsive control (Congdon et al., 2008). From an equine perspective, impulse control will be an important behavioural facet during many handling applications, where the animal may be required to suppress a flight reaction. However, as the temperament trait of impulsivity is itself multifaceted (including impaired response inhibition and response to novelty), so this must be considered when attempting to investigate the genetic origin of this behavioural trait. Nevertheless, individuals with a 7-repeat allele DRD4 polymorphism demonstrated significant impairment during a Stop Signal task thereby demonstrating impulsivity (Congdon et al., 2008). Once again this demonstrates the impact of dopamine receptor genetics on temperament traits that could provide insights into cognitive function through the development of both behavioural and genetic markers in the horse.

4.6. Genetic basis of stress coping strategy

For a gregarious herbivore, commonly applied horse management strategies such as restricted forage intake and movement, are often perceived as stressful, leading to activation of behavioural coping strategies, which in other species appear to be under a degree of genetic control. Much of the work to date features two inbred genetically distinct strains of mice, the DBA/2 and C57/b (Cabib, 2006). In response to stressors such as social isolation, the former deploys what is referred to as the active response, characterised by hyper-locomotion and stereotypy, whereas the latter features behavioural depression and reduced activity (Cabib et al., 2012). From a neural standpoint, the active strategy results from increased dopamine transmission into the nucleus accumbens, whilst the passive features decreased release of this neurotransmitter (Cabib and Bonaventura, 1997). Interestingly, horses with active coping tendencies appear to experience similar elevations in dopamine transmission, thus, there appears to also be a link between active coping individuals of this species and stereotypy predisposition (Bachmann et al., 2003). Moreover, recent behavioural probes of dopamine activity (spontaneous eye blink rate and behaviour initiation frequency) revealed strong evidence for elevated dopamine in horses performing both oral and locomotor stereotypy (Roberts et al., 2015). Taken together, it would appear that the neural correlates of active coping style at least, are common to both equine and murine systems. When considering issues of ethological survivability, for free living equids, the active style is likely to be more effective, especially considering the open expanses of space occupied by feral horses. In such a niche, behavioural depression in the face of predation stress would lead to death. However, for species relying upon stillness and camouflage in dense foliage the passive style is likely to be more appropriate. Furthermore, and through the ocular of domestic management, passive tendencies would be of potential benefit, even to a species like the horse due to the long periods of inactivity and movement restriction often imposed. As such, genetic probing of coping style warrants further research effort, in view of developing genomic tools of prediction. With this in mind, and given the linkage between stereotypy and active coping, Hemmann et al. (2014) investigated gene targets linked to crib-biting, including polymorphisms with close proximity to the D1 dopamine receptor gene (DRD1) and actual coding regions of the mu-opioid receptor gene (OPRM1). No associations were uncovered in this study, although more detailed analysis of coding regions of the DRD1 gene have revealed a cytosine deletion with an 80% prediction efficiency with regards to the crib-biting phenotype (Hemmings, 2012). The later study however, featured a small study cohort (n = 20) and so these data are of limited significance and further work is certainly required. Furthermore, the candidate gene approach to mutation detection has been superseded in recent years by techniques such as the Genome Wide Association Study (GWAS), which enables more holistic investigation of the genome. Undoubtedly, modalities such as this will offer significantly greater chances of uncovering genetic differences should they exist. Of course, should a genetic test for aspects of active coping ever be made available, consideration is warranted with regards to its application. This factor is particularly relevant to the production of horses for disciplines such as racing and eventing, which may partly rely upon active coping tendencies for competition success. For example, crib-biting and weaving animals were recently shown to acquire a simple operant learning task significantly faster than stereotypy free controls (Roberts et al., 2015). Furthermore, top riders positively select for active coping tendencies (Hemmings, 2011). Therefore, given the negative perception of stereotypy performance in the horse owning populace (McBride and Long, 2001), undue selection against active coping in an effort to banish stereotypy could lead to reductions in trainability and in turn performance.

4.7. Genomic predictors of aggression

The herd structure of feral equids features various mechanisms geared towards avoidance of con-specific aggression (Nunez et al., 2014). Furthermore, even in the face of predation, the horse evolved coping tools to favour flight rather than fight. That said, in the domestic setting some animals deploy inappropriate/unwanted instances of aggression that increase injury risk to handlers and other horses. Therefore, genomic predictors of aggression may be useful, particularly when animals are required for disciplines such as Equine Assisted Therapy (EAT) where unpredictable and threatening output from human subjects are commonplace.

Functional deletion of the tryptophan hydroxylase (TPH) gene from the murine genome leads to a hyper-aggressive phenotype (Mosienko et al., 2012) whilst polymorphisms of this locus lead to aggressive tendencies in humans (Zalsman et al., 2011). Tryptophan hydroxylase is the rate-limiting enzyme in the biosynthetic pathway responsible for the conversion of tryptophan into serotonin (a key neurotransmitter which plays a role in reward and emotional stability) (Carlson, 2001). Knockout strategies such as that undertaken by Mosienko et al. (2012) commonly lead to a 98% reduction in brain serotonin levels, a neurochemical imbalance that is believed to underlie the recorded increase in impulsive aggression. Building on findings such as this, Momozawa et al. (2005) initiated the search for naturally occurring TPH2 mutations in equine populations. This limited study found no significant association with polymorphic regions of this gene and aggressive tendencies, in agreement with other findings (Hemmings, 2012). Drawing once again from better studied species, polymorphic variants at a range of additional gene targets (including the D2 dopamine receptor [DRD2] locus) have been identified as contributory to hyper aggression (Butovskaya et al., 2013), and thus the search for equine gene targets should undoubtedly be widened accordingly, preferably via GWAS.

5. Conclusions and areas for future research

Understanding the mechanisms underpinning behaviour brings additional understanding about the behaviour itself. In particular, it provides a better awareness of how factors can affect behaviour, from the interaction of genes and environment, through to specific events such as the application of an aversive stimulus. Practically, this allows further optimisation of training techniques and the opportunity to improve the husbandry of the horse.

The neurotransmitter dopamine has featured heavily in this review. This reflects its pivotal role in the underlying mechanisms

of learning and how it directs and motivates the animal towards positive resources and away from potentially harmful aversive stimuli. Dopamine is also crucial in determining the day-today welfare of the animal. Up-regulation of dopamine, as a result of environmental factors such as nutrition or early life stressors, can produce a hyper-motivated phenotype. These individuals are often unable to attain their highly motivated goals due to the restrictive nature of the stable environment. This increases stress levels of the animals and is also the potential basis for stereotypy development. Down-regulation of dopamine, also as a result of stress, can lead to behavioural depression.

This review has highlighted the use of non-invasive methods for assessing dopaminergic tone in the animal as well as more general cognitive tests for monitoring specific psychological traits, such as impulsivity and behavioural inflexibility, that may be useful from a practical perspective. Many of the tests listed in Table 1 have been validated in the horse and thus provide the basis for future longitudinal testing to assess how husbandry factors affect the neuropsychological state of the animal. More importantly, it provides an empirical method to formally test how neurophysiological state (and behaviour) can be modulated through specific equine husbandry strategies, in a way that may be both beneficial to the animal's welfare as well as its performance ability.

Conflict of interest statement

None.

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None.

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