CAN DOPAMINERGIC TONE BE MEASURED INDIRECTLY TO ASSESS THE WELFARE OF THOROUGHBREDS?

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INTRODUCTION: SBR AND BSF

SPONTANEOUS BLINK RATE (SBR)

Spontaneous blink rate is thought to be an indirect measure of dopaminergic tone in the CNS.

SBR is defined as the rate of bilateral paroxysmal brief repetitive eye closures, occurring continuously and in the absence of obvious external stimuli (Karson, 1983). In humans it serves as a reliable though non-distinctive method of assessing dopamine function, being preferable to invasive and expensive techniques (Jongkees & Colzato, 2016).

SBR AS AN INDIRECT MEASURE OF DOPAMINE PREVIOUS RESEARCH

- Karson et al, carried out a study in 1983 in which a dopamine agonist; apomorphine was administered to four Rhe- sus Monkeys. A four-fold increase in SBR was observed which was then abolished following injection of the dopamine antagonist sulpiride; elevated dopamine within the CNS showed to increase SBR and dopamine suppression decreased SBR.
- Further investigation into a positive relationship between SBR and dopamine was investigated in equines with Cushing's syndrome, which causes neuro-degeneration of dopamine neurons, research has shown that sufferers have a significantly depressed SBR (Mcbride, 2017).
- Further research has been carried out on both humans and animals, illustrating similar findings, in particular Andrew Hemmings' work on equines, which I will address throughout.
- SBR is believed to measure dopamine levels in the Dorsal Striatum; as SBR is thought to be controlled by the Substantia Nigra in the midbrain and terminating in the Dorsal Striatum (Karson, 1983).

BEHAVIOURAL SWITCHING FREQUENCY (BSF)

Behavioural switching frequency like SBR is also thought to indirectly measure dopaminergic tone.

BSF measures the rate of switching from one behaviour to another (Garner, 2006). Measurements can provide additional insights into dopamine function at the level of the Striatum. On administration of a dopamine agonist; an increase in BSF was measured, like in SBR (Robbins et al, 1983). Thus, it is thought to also be an indirect measure of dopaminergic tone and since dopamine is high in stressed horses, has the potential to be a tool that might measure habit formation in equines (McBride et al, 2017).

AIM

To asses whether SBR and BSF could be used to measure the welfare of racehorses, by indirectly measuring dopamine levels and in doing so assess whether they are stressed or anxious and if they may be predisposed to stereotypic behaviour as a result of high stress.

HYPOTHESIS

We would expect to see an increase in SBR in horses with higher dopamine levels as a result of either stress or rewarding tasks such as feeding and a lower SBR in more relaxed equines.

BSF would be expected to increase with increased dopamine levels, therefore we would expect to see a higher BSF in more stressed or anxious equines.

Oral stereotypy's have shown to have abnormalities in the brain; causing lowered dopamine levels, so we would expect to see lowered SBR.

The project I have undertaken has been a smaller part of a project taking place at Langford, University of Bristol. Where they are collecting data in order to make a welfare assessment tool, that can be used by inspectors on racing yards. There is currently no way in which to measure the welfare of racehorses when at home. This will hopefully improve the welfare of horses at home and on the track. Their project is sponsored by the racing foundation and the BHA.

Data from each of the yards has been collected at the beginning and the end of the flat and national hunt season, in order to assess any changes in temperament and so welfare. My visits took place at the end of the flat season, therefore the horses I measured had already been measured previously and assessed for stereotypic behaviors. Due to the nature of race yards many of the stereotypic horses that I had planned to assess had moved to other yards or gone from the yard for another reason. Therefore I was only able to assess the horses that were still on the yard.

Andrew Hemmings' data and work is referred to throughout, as he has previously completed work on SBR and BSF in horses. I met with Andrew at the beginning of my project to discuss important aspects that should be considered and how to collect my results.

BHA
BRITISH HORSERACING AUTHORITY

THE
RACING
FOUNDATION

METHOD

How many yards were visited and how long for:

Three flat racing yards were visited for two days each.

How the horses were selected:

All horses that had previously shown stereotypic behaviour were selected to be measured (n=3). For each of these a non-stereotypic horse (n=26) in a neighboring stable was chosen or a horse of the same age and gender. At least two horses from each sex and each age group were chosen, these were stabled near to each other and measured at a similar time of day, this was to ensure the environment was same.

How many times each horse was measured:

Each horse was then measured at least twice at different times of day to see whether the environment had an effect on the results.

Before taking the measurements:

I noted down the horses name, sex, age, as well as; how busy the yard was using the busyness score system from 0-3, the noise on the yard at the time in dB-A and the date and the time, which were vital to understand if the horse had recently been ridden, if it was near feeding time or if it was the afternoon and very quiet.

How each horse was measured:

Each horse was measured for 30 minutes, in 5 min blocks for half blinks, full blinks and BSF . After 5 mins the measurements were noted down including notes on what behaviors took place, how the horse seemed and anything that may have affected the results during those 5 mins ie. if the horse was stood in direct sunlight or if other horses returned from the gallops halfway through measurements.

What conditions were the horses measured in:

When taking the measurements the horse was free in its box, I stood outside the box as far out of sight as possible whilst still being able to view the horses eyes.

EXTERNAL FACTORS

SBR external factors to consider for increased blinking:

- Dry eye
- Light
- Wind
- Temperature
- Disease of the eye
- Dust particles

When considering spontaneous blink rate, it is important to consider all other factors and what role they might or do play in increased blink rate of a horse. There is no text on the average blink rate of a horse per minute, however in humans it was found around 3-25 blinks took place per minute (Briggs et all, 1999).

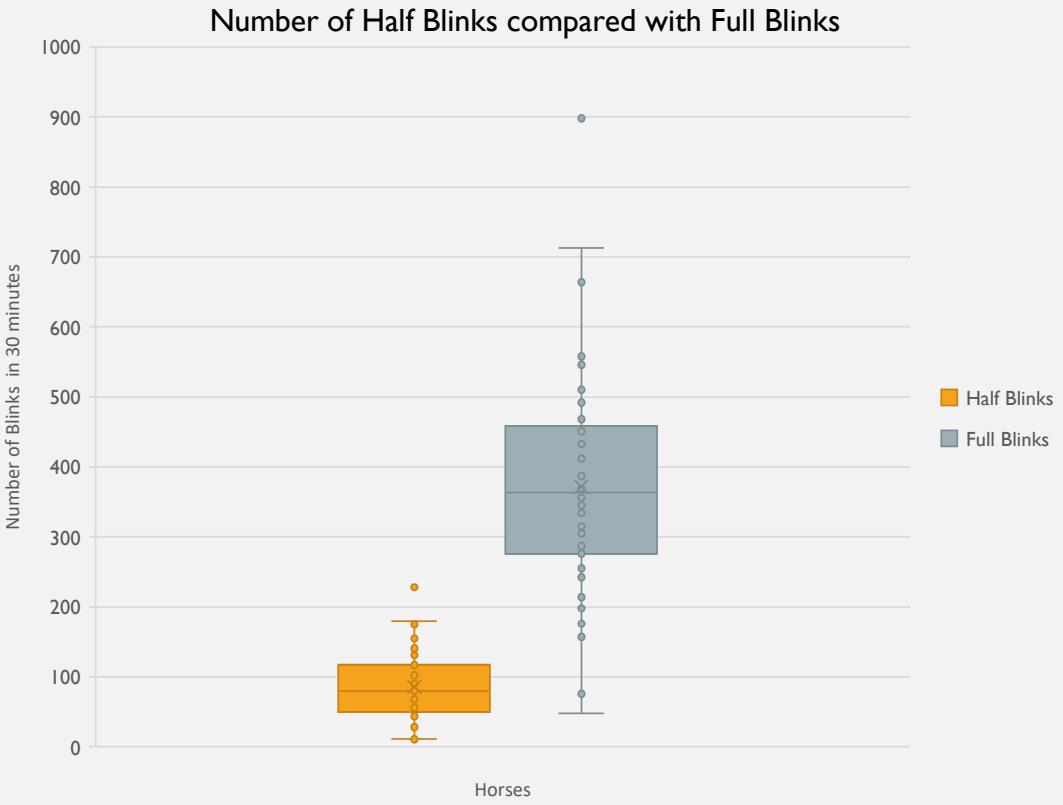
How this was managed:

- Light intensity was measured where applicable, and noted down.
- Noise was measured using a Db-A meter each time.
- If it was particularly dusty or windy this was noted
- No horse was measured that had any sort of disease of the eye.
- Time of day was noted and what this meant for the horse ie feeding, just come back from the gallops, or lunch time so very quiet etc.
- Busyness score was also used to note down the busyness of the environment at the time of measurements.

Busyness score:
0 = no one else around
1 = very calm, one person around
2 = a few people around talking / tacking up/ mucking out
3 = lots of people about or feed time/ back from gallops

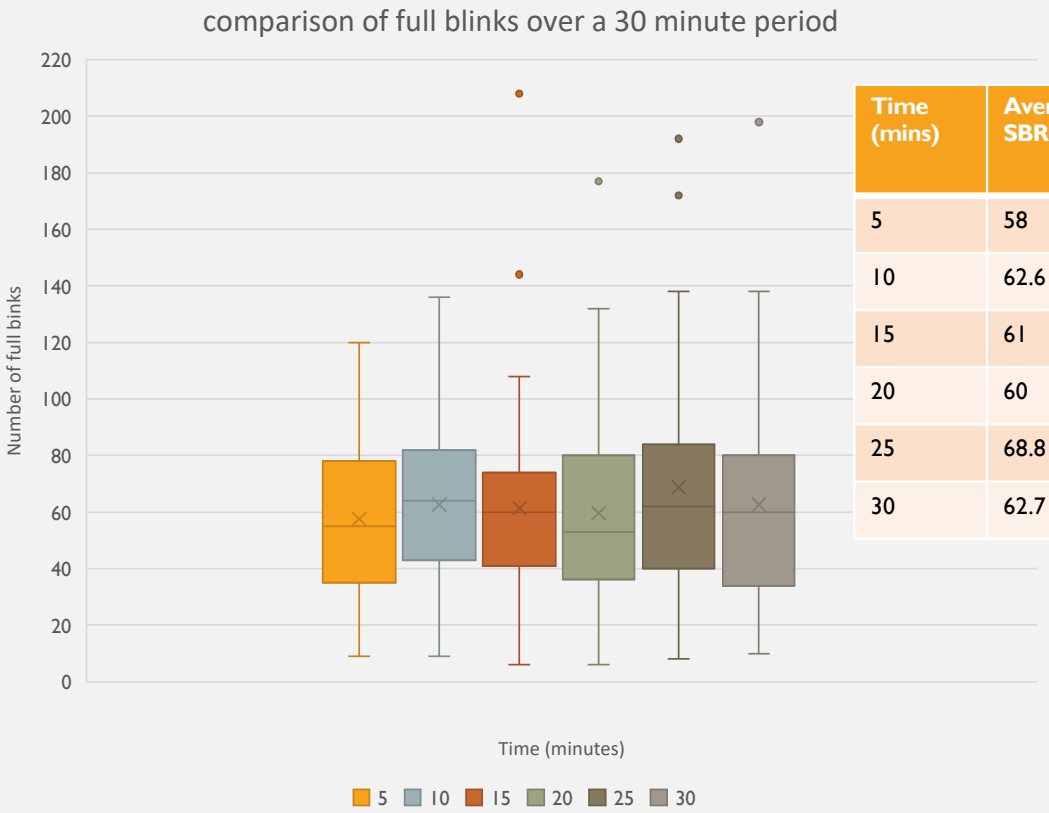
RESULTS

COMPARISON OF FULL AND HALF BLINKS



Half blinks have not previously been accounted for in other studies, therefore it was important to measure them to see whether they were of importance. Half blinks amounted to 18% of the amount of total blinks and were roughly only 22% of the amount of full blinks across all horses. For this reason, they seemed to have no relevance to the data. Only two horses showed an increased rate of half blinks, but were not included in the average as they were in direct sun light on a particular sunny day when being measured. For this reason half blinks have not be analyzed any further.

COMPARISON OF SBR OVER TIME

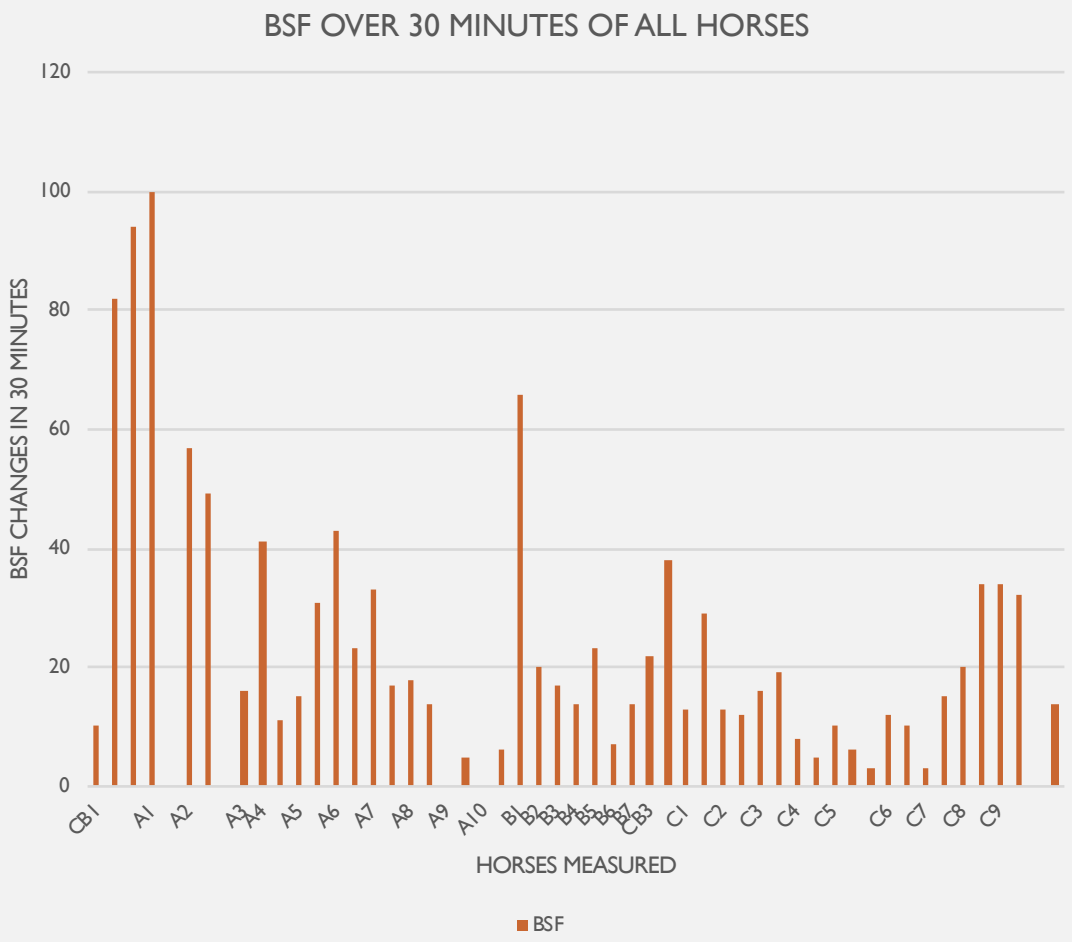


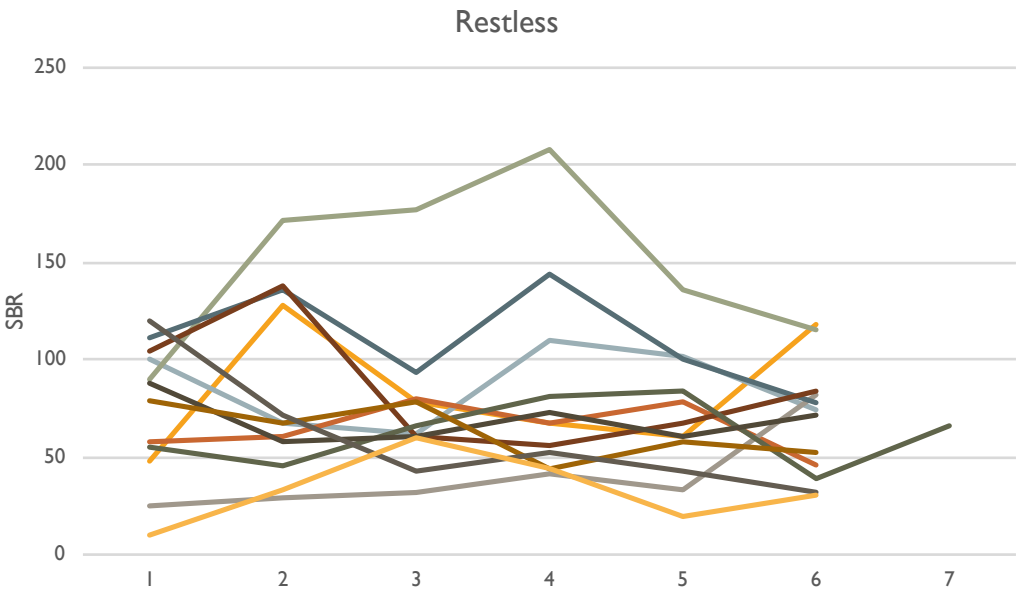
Time (mins)	Average SBR	Standard deviation
5	58	27
10	62.6	29
15	61	34
20	60	34
25	68.8	40
30	62.7	36

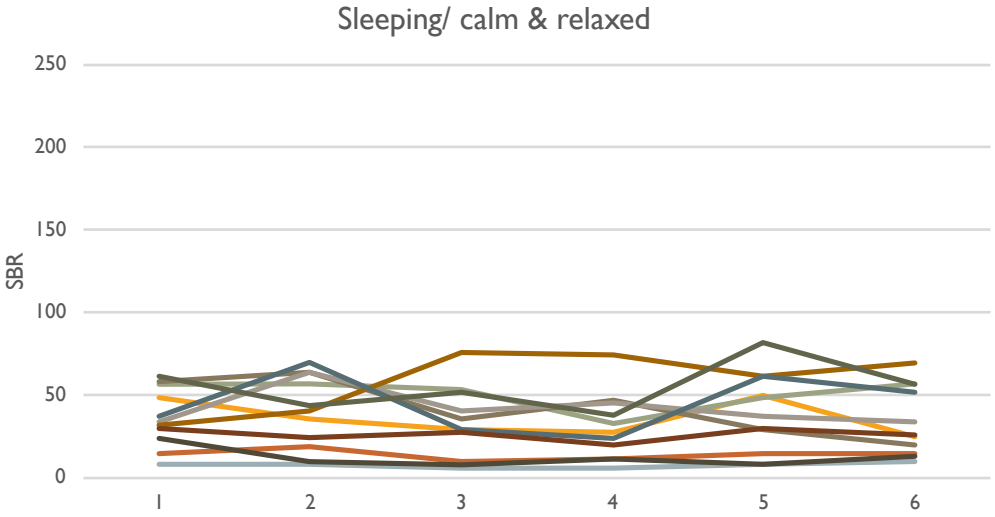
Over a 30 minute period, it was important to consider whether the full 30 minutes was necessary for comparison when analyzing the data. From this data I decided to account for the entire 30 minutes, as there is not a huge amount of variation throughout, therefore the total SBR in this time has been used. Though, in some cases its important to see the variation across the 30 minutes especially around feeding time, therefore where this is important this data has been shown.

BEHAVIOURAL SWITCHING FREQUENCY

Equines were accounted as restless if they had a behavioural switching frequency of more than 25. This is because they were either showing signals of being anxious or were stood with their ears forward looking over the door, or out of a window constantly. In the data there is a positive relationship between restlessness and BSF, which may indicate that high stress is linked with high BSF.





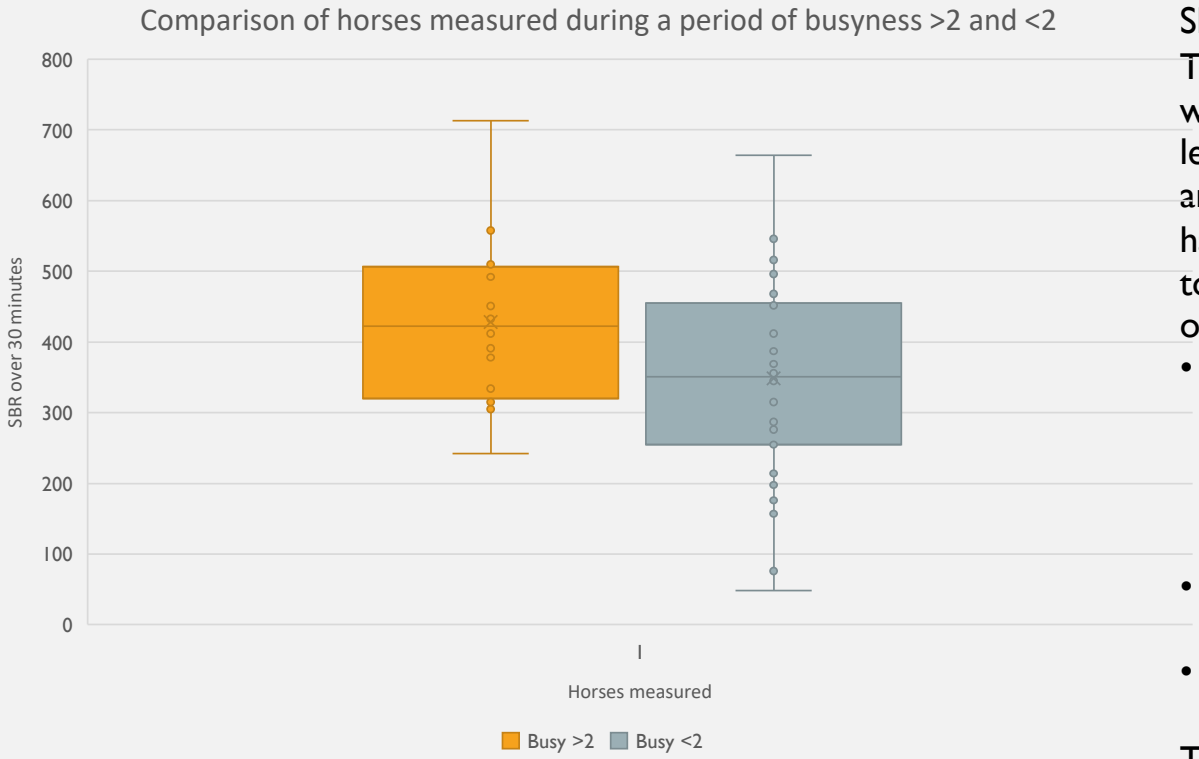


SBR & BSF RELATIONSHIP

Equines with a score of more than 25 BSF within 30 mins were presented as restless and the rest of the horses were classed as sleeping/ calm. From this data there is an increase in SBR in restless horses, though most notifiable is the range of SBR recorded across the 30 minutes compared to the "relaxed" horses. This gives evidence that BSF has a positive relationship with SBR and so may also indicate an increased level of dopamine in restless horses.

Mean SBR:
Restless horses: **433.92**,
Sleeping/ calm horses: **346.66**

EFFECT OF BUSYNESS OF YARD



From the graph we can see there was a general increase in SBR during periods of busyness of 2 or more.
This must also be considered for each set of data and whether this has affected the behavior and potential stress levels of the horse. A busyness of 2 or more, in which there are a few people about talking or making noise, or horses have just come back from the gallops, would be considered to be distracting for some horses and potentially excite them or cause them stress.

- 54.55% of horses showing restless/anxious behavior did so in a period of time where the yard was at least a score of 2 for busyness. This is 46.15% of the number of horses that were measured during a period of busyness of 2 or more.
- 39% of TB's showed anxious/stressed behavior in at least one reading.
- 36% of these (14% of the total) showed anxious behavior during a busyness score of 2 or more and less than 2.

Therefore the other 64% we must consider have been affected by another factor such as; environment, time of day, busyness of the yard or feeding time.

ORAL STEREOTYPIC EQUINES

ORAL STEREOTYPY'S HYPOTHESIS

GASTRIC HYPOTHESIS:

There are multiple hypothesis around oral stereotypies, why and how they have begun and how this reflects the welfare and/ or the husbandry and welfare of the horse.

Research carried out on crib biting equines found a majority to have gastric inflammation, making them more susceptible to having gastric ulcers (Nicol et al, 2001).

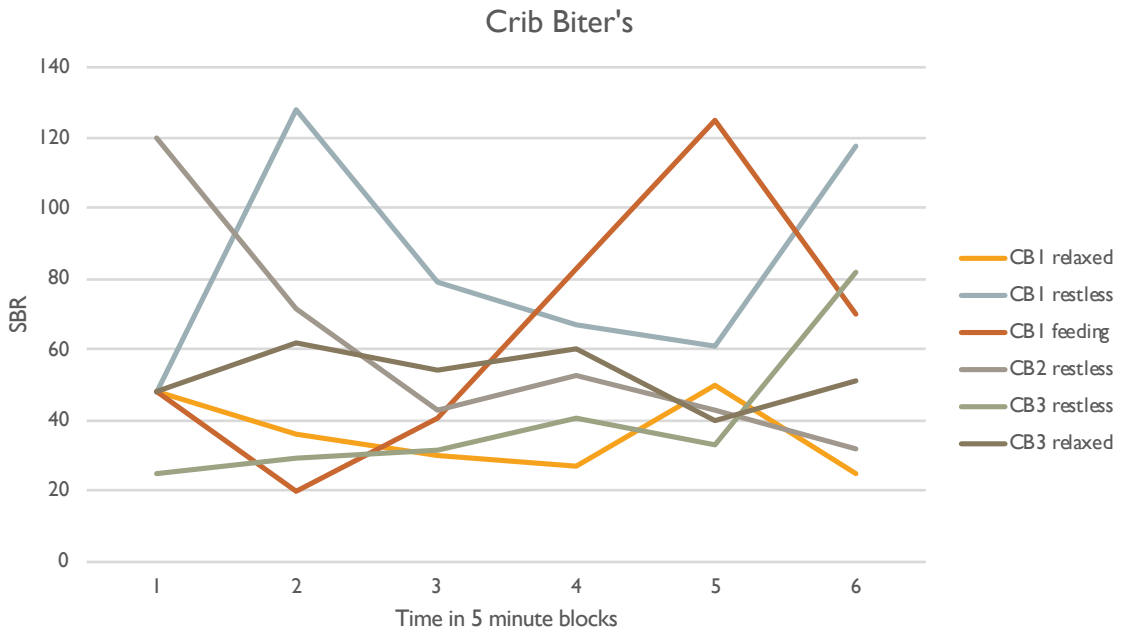
Upon further examination by Nicol et al, it was found that Crib Biters produce more alkaline saliva, which is required for gastric buffering. In the wild horses eat for 16-18 hours and produce 35-40L of alkaline saliva each day. Thus it was hypothesized that crib biting is an attempt to replicate mastication and produce saliva to reduce gastric pain (Roberts et al, 2017). Racehorses in particular are eating for far less time, thus producing less saliva and so in accordance with this theory would be more susceptible to gastric ulceration and so crib biting. Further research which would support this theory has shown that feeding anti-acids and or feeding more regularly to Crib Biters has reduced their crib biting behaviour (Mills and MacLeod, 2002).

DOPAMINERGIC HYPOTHESIS:

Hemmings et al, counter the gastric argument and state that; 'stereotypic behaviors are often a result of a highly stressful event early in life or by frustration, creating a horse with a highly motivated behavior phenotype, whom requires more positive stimulus and whom then creates a coping mechanism: oral stereotypic behavior (Roberts et al, 2017)'. If SBR and BSF can accurately measure dopamine levels, the tests would help us to identify horses who may later develop stereotypic behaviors, due to currently being in an environment that they find highly stressful.

There are a number of texts pointing towards different reasons as to why oral stereotypies evolve, one thing that can be concluded from each of these results is once the oral stereotypic behavior begins, it is almost impossible to stop it as it becomes a habit. There are methods used such as surgery or physical barriers such as collars, though these mechanisms have shown to increase stress, as the horse is no longer able to exert its coping mechanism (Nagy et al, 2009). Therefore preventing the stereotypic behavior from starting is the only way to stop it. This could potentially be established by using a simple test such as SBR or BSF that could prevent stereotypic behaviour's from developing, by recognising high stress early on.

SBR AND ORAL STEREOTYPIES

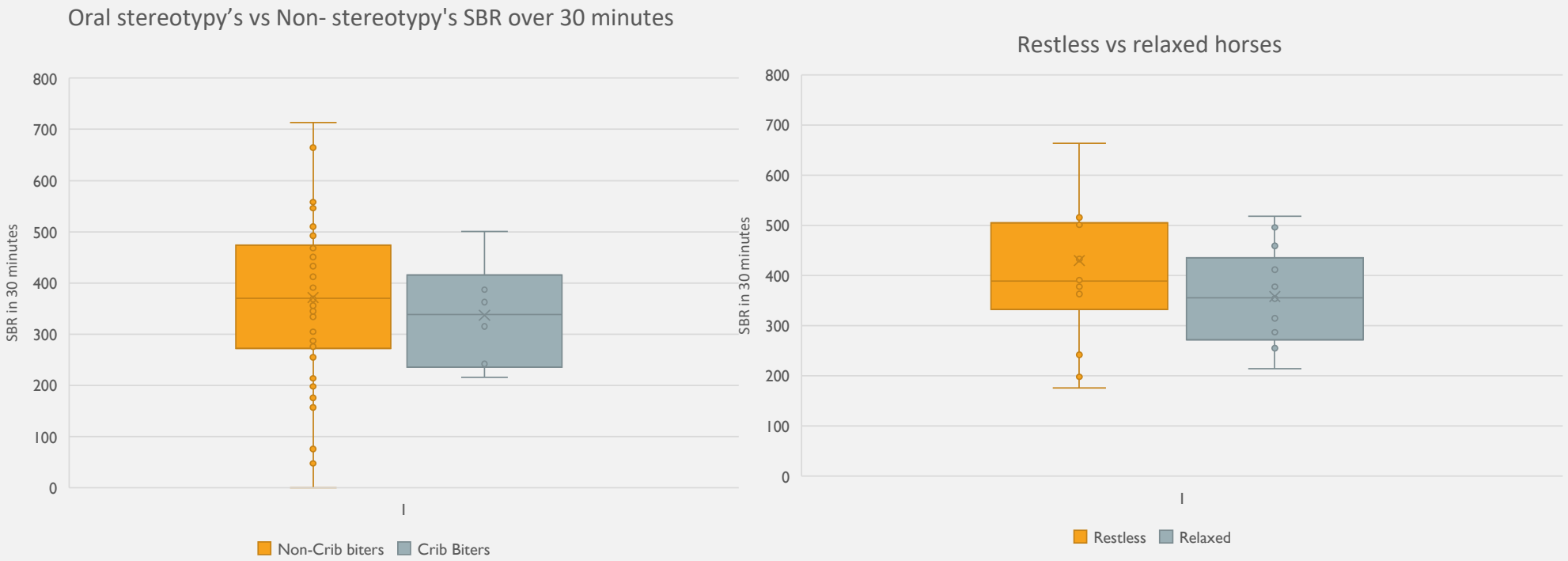


Time (mins)	09:54, busyness =2-3	13:01, busyness = 1
5	Standing/ chewing bars/ head over door	Head over door
10	"Head over door- blinked more, scraping teeth on bars, eating hay, walking around box	CB/ eating hay- very calm
15	CB/ eating	"
20	Horse came back from gallops-3, head over door & chewing bars	sleeping
25	Busy-3, CB- low blinks during, increased blinks after CB	CB/ eating
30	Busy-3	CB/ eating

This table shows the notes from CB3 at 5 minute intervals during two different measurements.

Previous research carried out, has noted that Crib Biter's (CB) in fact showed a decreased SBR compared to 'normal' horses. It is thought this is because crib biting has been established as a coping mechanism and therefore when the horse cribs it is destressing itself and therefore possibly more 'calm' than a horse that has not established a coping mechanism (Hemmings et al, 2015). Nagy et al, also found that crib biters demonstrated a significantly lower 'anxiety' score. This is evidenced with CB3, whom I initially observed on 3 occasions, however there was little difference between SBR, the time of day, feeding time or after being ridden, SBR of CB3 was also very low in comparison with non-stereotypic horses.

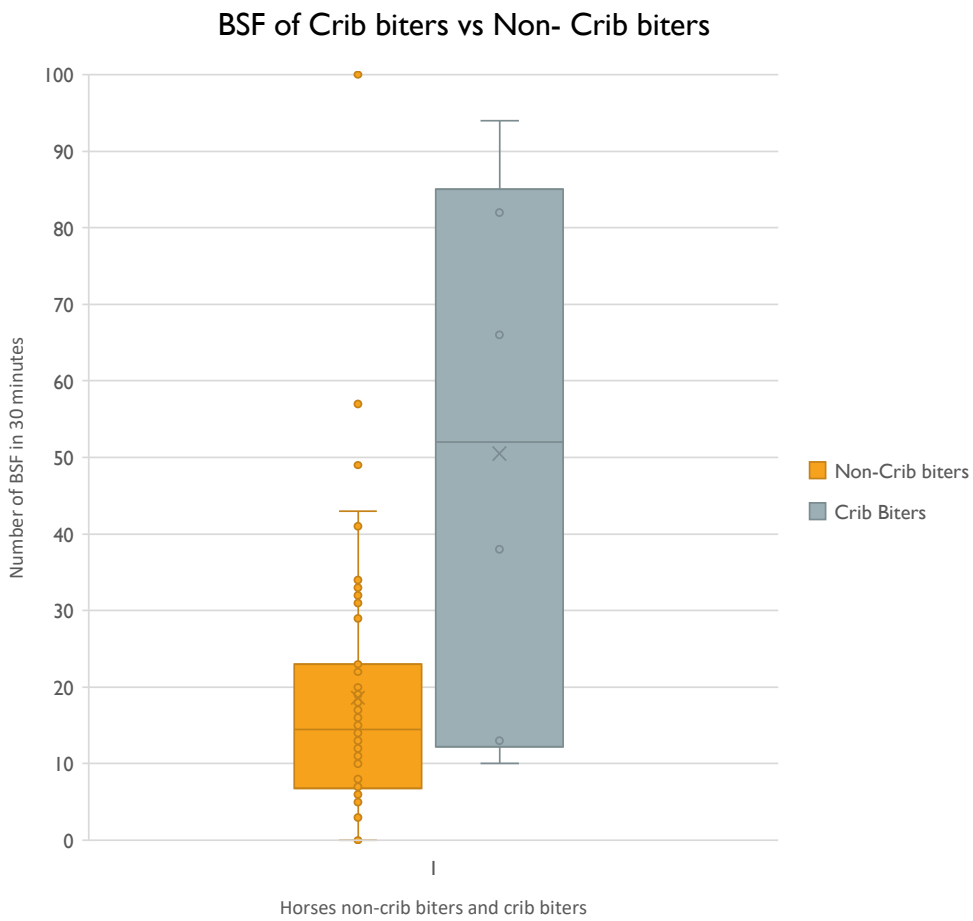
STEREOTYPIC VS NON-STEREOTYPIC



Furthermore, non-stereotypic horses have a much wider range of results of SBR and are generally higher than the oral stereotypic horses. When compared with the results of 'Restless vs Relaxed horses' the two graphs are very similar, with the relaxed horses having a very similar, though slightly higher results compared to the crib biting horses.

BSF AND ORAL STEREOTYPY'S

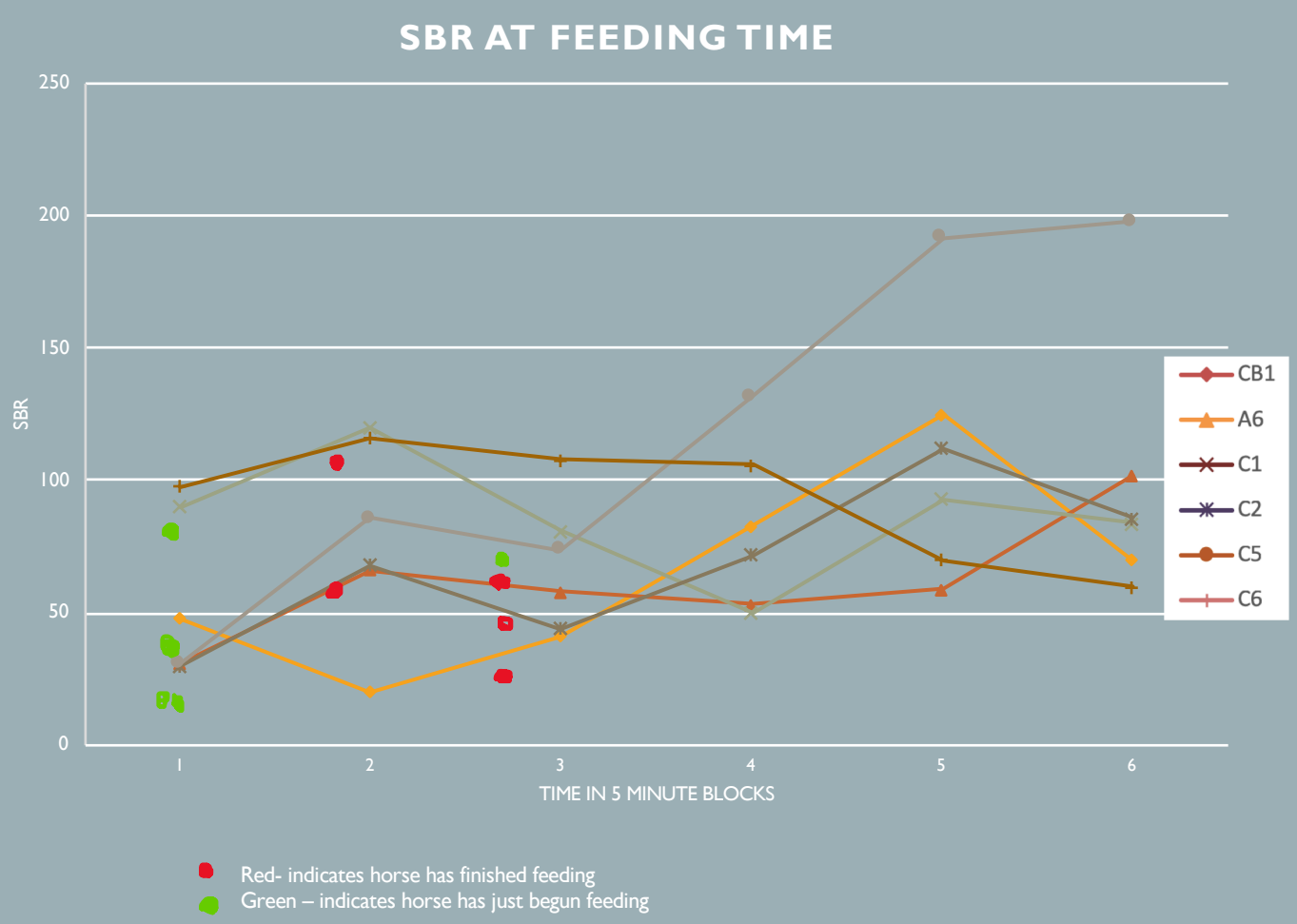
From the results we can see there is a large range of results for Crib Biter's and BSF and a significant increase of BSF when compared with non-stereotypy's. This may suggest that there is a positive relationship between BSF and Crib Biter's. Roberts et al, also found this in their research and suggested that BSF is indirectly measuring dopamine levels in a different part of the CNS to SBR, since there is a decrease in BSF yet an increase in SBR in oral stereotypy's.



ORAL VS LOCOMOTOR STEREOTYPIES

There were no race horses presenting with locomotor stereotypic behavior on the yards visited, so data could not be collected on this occasion. However, previous research by Hemmings et al, has looked at both oral and locomotor stereotypy's and found that locomotor stereotypy's presented with a significantly higher SBR compared with Crib Biter's and a slight increase in SBR compared with non stereotypic horses. They also found locomotor stereotypy's had a lower BSF compared to Crib Biter's but higher than non-stereotypy's (Hemmings et al, 2015). This data might suggest that weaving increases dopamine levels in the CNS, compared to crib biting which decreases dopamine levels overall.

SBR AND FEEDING TIME



CB1- Shows a definite increase then finally a decrease: Finishes hard feed at 15 mins, switches between eating and CB then resting with head over door once finished.

A6- Shows a more gradual increase: Finishes hard feed at 15 minutes, then foraging around feed bowl and looking alert.

C1- Hard feed is given at 15 mins (3), increase before feed given in anticipation and and increase again after feed is given.

C2 – Increase throughout: finishes hard feed at 10 minutes, then foraging in bedding for hay.

C5- Finishes hard feed and moves onto hay at 15 minutes, shows a large increase.

C6 – Finishes hard feed at 5 minutes, then resting with head over door and licking lips after this point.

There are many texts that evidence that eating increases dopamine levels; dopamine metabolism was activated in post mortem in the animal brain following feeding, suggesting that dopamine release was increased by feeding (Blackburn et al, 1986).

In this data, all horses except from C6 show an increase in SBR after feeding, this is in line with a study on rats carried out by Yoshida et al, in which they found dopamine levels increased more after eating and remained elevated for 20-60 minutes afterwards. Since we know dopamine is increasing at this time, this is further evidence that SBR is increasing as dopamine is increasing, showing a positive relationship between the two. Thus, amplifying the possibility of SBR being used as a welfare tool indicator.

CONCLUSION

SBR & ANXIETY IN EQUINE

- **Anxious equines have elevated dopamine levels when compared to less anxious horses.** Common causes of anxiety in animals can be caused by feed restriction and social isolation; both of which are very prevalent in racehorses. Therefore we might expect a higher proportion of racehorses to have elevated dopamine levels and so a higher SBR and BSF.
- However, upon comparison with the mean results collected here and with those results collected by Roberts et al, 2016 (in there research neural modulators of temperament) they found over a 30 minute period their mean for SBR to be: 547.72 and BSF mean to be: 24.94, this included pony/cobs and sport horses. Conversely, the racehorses mean results collected in this study were considerably lower; 370.86 for SBR and 23.2 for BSF. This may suggest that in fact racehorses have lower dopamine levels overall which could be an indicator of good welfare, as whilst racehorses are fed less and do not have interactions in the field, they are all cared for to high standard's.
- For the results it is important to consider that only some of the best yards came forward to allow data to be collected, these yards wanted a welfare assessment to be carried out and were keen to hear the results. Thus, we would expect that they care highly about their horses and so already give them the best and most appropriate care that they can. This may support the theory that these racehorses have better welfare overall and is the reason for a lowered SBR and BSF compared to the other results.
- However, it is important to consider differences in methodology used by Roberts et al, in which; horses were tied up in their stable and each measured for a solid 30 minutes without stops, which could of course contribute to the difference in results.
- Overall, SBR has shown to increase in horses that appear restless and stressed, this could indicate towards higher dopamine levels. One of the horses measured on one race yard had a very high SBR and BSF, during the second measurement taken of him he started to weave. This may indicate that his stressed behaviour is already getting to the point in which he is seeking coping mechanisms through stereotypic behaviours.

FROM THIS DATA WE CAN CONCLUDE:

- There is no link between half blinks and full blinks from these results, so half blinks have been ignored.
- There is a link between BSF and SBR and from this, a conclusion has been made to treat any equine with more than 25 BSF as suffering from stress or anxiety.
- BSF and SBR produced opposite results when measuring oral stereotypic horses, we might conclude that they are in fact indirectly measuring dopamine levels in different parts of the Striatum.
- There was a positive relationship between anxiety and SBR and a negative relationship between docility and SBR, this has been concluded not only from these results but the results of Roberts et al.
- Busyness and environmental factors were shown to increase anxiety in some horses as during calmer times they were much less anxious, although 14% of the total of horses measured showed anxious behaviour even when the yard was calm.
- Oral stereotypic horses showed decreased SBR and potentially decreased dopaminergic tone, this is most likely because they have developed coping mechanism which allow them to cope with their stress and remain calm.
- A positive relationship exists around feeding time and SBR, this is important as feeding is known to increase dopamine levels (Blackburn et al, 1986).

CAN SBR AND BSF BE USED AS WELFARE TOOLS?

Horses presenting with constantly increased SBR and BSF are horses that have shown to be stressed and anxious. High stress means high dopamine levels, high stress is also directly associated with lowered welfare. Given the previous links and evidence we might therefore conclude that it is possible to indirectly measure dopamine levels via SBR and BSF and thus enabling us to use them both as a welfare tools.

BSF in particular could be used to measure habit formation and impulsivity, which could be a useful marker for stereotype disposition, accelerated and/ or impulsivity (McBride et al, 2017).

In order for this conclusion to be made we must first collect further data, including into the real cause of stereotypic behaviours and secondly in particular must submit the horses aforementioned, to a change in husbandry or routine in some way that decreases their stress levels and then again measure these horses to see if there is a change and so establishing if this was the cause. If that is successful in a number of cases this might give more accurate data, pointing towards SBR and BSF as welfare tools.

If SBR and BSF could be used as tools to indirectly measure dopamine, we are able to simply and easily measure the stress of a horse and manage that horse in a way which better suites them. Though further data must be collected in order to affirm this conclusion.

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