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Evidence of negative affective state in Cavalier King Charles Spaniels with syringomyelia

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Highlights

• Dogs with syringomyelia (SM) show a more negative judgement bias than those without SM
• SM dogs do not show a greater sensitivity to reward loss than SM-free dogs
• SM dogs scratch more than SM-free dogs but do not differ in jump up/down tests
• SM may be associated with negative affect in Cavalier King Charles Spaniels
Abstract

Syringomyelia is a common and chronic neurological disorder affecting Cavalier King Charles Spaniels. The condition is putatively painful, but evaluating the affective component of chronic pain in non-human animals is challenging. Here we employed two methods designed to assess animal affect – the judgement bias and reward loss sensitivity tests – to investigate whether Cavalier King Charles Spaniels with syringomyelia (exhibiting a fluid filled cavity (syrinx) in the spinal cord of ≥2mm diameter) were in a more negative affective state than those without the condition. Dogs with syringomyelia did not differ in age from those without the condition, but owners reported that they scratched more \((P<0.05)\), in line with previous findings. They also showed a more negative judgement of ambiguous locations in the judgement bias task \((P<0.05)\), indicating a more negative affective state, but did not show a greater sensitivity to loss of food rewards. These measures were unaffected by whether the dog was or was not receiving pain-relieving medication. Across all subjects, dogs whose owners reported high levels of scratching showed a positive judgement bias \((P<0.05)\), indicating that scratching was not directly associated with a negative affective state. Tests of spontaneous behaviour (latency to jump up to or down from a 30cm high platform) and physiology (thermography of the eye) did not detect any differences. These results provide initial evidence from the judgement bias task that syringomyelia may be associated with negative affect in dogs, and open the way for further and larger studies to confirm findings and investigate the effects of medication in more detail.

Keywords

Animal welfare; Cognitive bias; Reward loss sensitivity; Affective state; Dog; Syringomyelia
1. Introduction

Syringomyelia is a neurological disorder commonly affecting Cavalier King Charles Spaniels (CKCSs) (Parker et al., 2011, Rusbridge et al., 2006). It involves the formation of syrinxes (fluid filled sacs) in the spinal cord, secondary to an obstruction in the flow of cerebrospinal fluid (CSF) (Rusbridge et al., 2006). In CKCSs, this is usually due to a Chiari-like malformation which is a developmental change to skull and cranial cervical vertebrae morphology characterized by rostro-caudal bony insufficiency (Rusbridge 2004). A consequence is that the brain and cervical spinal cord are overcrowded in the skull, especially at the cranio-cervical junction, leading to obstruction of the foramen magnum and CSF channels. These obstructions to CSF flow are thought to play a critical role in the aetiology of syringomyelia (Cross et al. 2009; Cerda-Gonzalez et al. 2009; Knowler et al. 2017a,b). In an MRI study of asymptomatic CKCSs, 46% were found to have syringomyelia upon MRI, rising to 70% in dogs aged six years or older (Parker et al., 2011).

Syringomyelia in dogs is thought to cause chronic neuropathic pain (Rusbridge et al., 2006). Reported clinical signs that may indicate pain include frequent scratching of the caudal head and neck area. However ‘phantom scratching’ towards one shoulder or neck region without skin contact is not necessarily associated with pain (Nalborczyk et al., 2017). Other signs include spinal hyperaesthesia (aversion to being touched especially in the cervical and thoracolumbar regions) and vocalisations resembling “screaming” after sudden head movements, when rising, and when the dogs is lifted under the sternum (Rusbridge and Knowler, 2004). Syringomyelia also occurs in humans, often as the result of a Chiari type-1 malformation similar to that seen in dogs (Todor et al., 2000). 50-90% of human patients report pain as a prominent feature (Todor et al., 2000), with around 40% reporting unpleasant burning, tingling or stretching sensations (Milhorat et al., 1996) that are often “overwhelming and pervasive” (Todor et al., 2000). Similarities in pathogenesis between humans and dogs with syringomyelia, the fact that pain is a central characteristic of the disease in humans, and the nature of the spontaneous behavioural signs seen in dogs, strongly suggest that syringomyelia can be painful in this species. However, not all dogs show these
behaviours, even when MRI scans indicate the presence of syringomyelia (Parker et al., 2011), so questions remain as to whether, for example, these dogs are in pain despite not exhibiting any signs. Measures designed to assess affective state may help to address these important uncertainties.

Assessing the affective experience of pain in dogs, or any other non-human species, is far from straightforward because ultimately we cannot be certain about the private subjective experiences or feelings of such species (e.g. see Paul et al., 2005, Mendl et al. 2010). Even in humans we have to rely on the indirect measure of linguistic report as our ‘gold standard’. Nevertheless, if we take the ‘componential view’ that affective or emotional states comprise subjective, behavioural, and neurophysiological elements (e.g. Paul et al., 2005), we are able to measure the latter two components objectively. Many current methods of pain assessment in animals, such as nociceptive threshold testing and reflex responses (Mogil et al., 1999, Roughan and Flecknell, 2001, Sneddon et al., 2003) focus on the sensory and nociceptive aspects of pain (i.e. the detection and encoding of nociceptive stimuli) and how these change in chronic pain conditions (Mogil 2009), rather than the affective component (i.e. the impact of the noxious stimulus on the animal’s emotional state). In clinical practice, pain assessment in dogs is often performed via subjective observation of, or validated scoring systems for, spontaneous behavioural signs thought to be associated with pain (Firth and Haldane, 1999, Brodbelt et al., 1997, Mathews et al., 2001). However, it is unclear whether the observed variability in propensity to display such behavioural signs (Firth and Haldane, 1999) is due to genuine variation in pain experienced, or whether some dogs are merely less likely to display behavioural signs than others.

Measuring the affective component of pain in chronic conditions such as syringomyelia is thus challenging (Mogil and Crager, 2004) but important. Here, we employ two measures that have previously been used to detect changes in animal affective valence (positivity/negativity): judgement bias and reward loss sensitivity. The judgement bias paradigm provides an empirical proxy measure of affective valence by assessing an animal’s interpretation of an ambiguous cue (Harding et al.,
It is based on findings from human psychology studies (Paul et al., 2005) and theoretical arguments (Mendl et al., 2010a) that individuals in a negative affective state are more likely to make negative (‘pessimistic’) interpretations of ambiguous stimuli than those in a more positive state, and has successfully detected negative judgement biases in conditions likely to induce negative affect in species including rats (Harding et al., 2004; Burman et al., 2008a; Enkel et al., 2010; Papciak et al., 2013), sheep (Doyle et al., 2011), pigs (Murphy et al., 2015), humans (Paul et al., 2011; Schick et al., 2013; Iigaya et al., 2016) and dogs (Mendl et al., 2010). There is also evidence in dogs that positive judgement biases occur following manipulations designed to induce a more positive affective state (Kis et al., 2015; Karagiannis et al., 2015). In a study of calves, negative judgement biases were seen between 6-22h after disbudding, which is likely to be painful and by which time the effects of local anaesthesia would have worn off (Neave et al., 2013). Here we use the paradigm to investigate whether negative judgement biases are observed in CKCSs with syringomyelia.

We also use a reward loss sensitivity paradigm. Unexpected omission of an expected reward is known to cause behavioural and physiological changes in a wide range of mammalian species (Papini and Dudley, 1997, Papini, 2003), and it is known that humans in a negative affective state show increased sensitivity to loss of reward (Rolls, 2016). Human patients with depression showed increased error-related negativity (brain event-related potentials that occur after an error is made) compared to healthy controls (Chiu and Deldin, 2007), as did people with greater negative affect as assessed by questionnaire (Hajcak et al., 2004). An animal’s sensitivity to loss of reward can be measured using the successive negative contrast method (SNC; Flaherty 1999) by training it to run to a point at which it receives the reward, and then unexpectedly decreasing the amount of reward given. Burman et al. (2008b) found that rats raised in an enriched environment but then housed in a barren environment showed a more prolonged response to the unexpected decrease in food reward (their latency to approach the low reward remained higher for more successive trials) than rats raised and housed in an enriched environment, suggesting that removal of enrichment induced an increased sensitivity to reward loss indicative of a negative affective state. SNC effects have been
demonstrated in dogs (Bentosela et al., 2009, but see Reimer et al., 2016) but without studying the effects of putative background affective state on response to a loss of reward. Here we employ a runway task similar to that used for rats to assess whether dogs with syringomyelia show a stronger slowing response to reward loss than control dogs.

We also use tests of physiological change and spontaneous behaviour that may provide further information about nociceptive and/or affective changes. We measure eye temperature as this has previously been used as an indicator of acute pain in other species. Stewart et al. (2008) found that calves dehorned without local anaesthetic initially displayed an initial transient decrease in eye temperature followed by a prolonged increase. Sheep showed increased eye temperature following ischaemic damage to the forelimb (Stubsjøen et al., 2009), and elk showed increased eye temperature following antler removal (Cook et al. 2006). If eye temperature measurement correlates with the presence of syringomyelia or with negative judgement bias, it offers a more convenient proxy measure of pain or distress. Additionally, since owners often describe a reluctance for dogs with syringomyelia to jump up or to climb stairs, we measure the latency for dogs to jump up to and down from a surface in exchange for a reward to assess whether syringomyelia affects the dogs’ mobility. We also use owner reports of frequency of scratching performed by dogs in their home environment in order to assess the severity of spontaneous behavioural signs of syringomyelia.

2. Materials and Methods

2.1. Animals

Ethics approval was granted by the University of Bristol, UIN number UB/12/010. 27 CKCSs were recruited using Clare Rusbridge’s website http://clarerusbridge-news.blogspot.co.uk/. Eligible dogs were purebred Cavalier King Charles spaniels that had had a MRI scan of the head and neck in the
last two years. Dogs that were known to have other medical conditions causing neurological signs, scratching or pain were excluded, as were dogs with grade III or greater mitral valve disease. It was not possible to exclude dogs with medication (e.g. NSAIDs, corticosteroids, opioid or gabapentin analgesics), since medical treatment is commonly initiated as soon as signs of syringomyelia become apparent. Neither was it possible to withhold medication during the study, as this may exacerbate the dogs’ pain or discomfort and thus would be ethically unacceptable.

Dogs were diagnosed with syringomyelia (SM) if their MRI results revealed a fluid-filled cavity (syrinx) within the spinal cord parenchyma with an internal transverse diameter greater than or equal to 2mm. Of the 27 dogs recruited, 11 were diagnosed with syringomyelia and 16 were free from syringomyelia. 11 dogs (7 diagnosed with SM on MRI and 4 diagnosed as free from SM) were on medication, and 16 (4 diagnosed with SM and 12 diagnosed as free from SM) were not taking medication. This discrepancy is probably because dogs may be put on medication due to behavioural signs of SM rather than following MRI, and around a quarter of dogs that display clinical signs of SM have no signs of a syrinx on MRI (Loderstedt et al., 2011).

Signalment data (e.g. age, sex, medication) and scratching scores as recorded on a Visual Analogue Scale (VAS) by owners, were collected via questionnaire prior to visiting the dog at its home. Data were collected in owners’ homes by AC and veterinary student Audrey Dupont in the following order: eye temperature recording, judgement bias testing, reward loss sensitivity testing, and jump up/jump down latency.

2.2. Scratching score

Owners were given instructions on completing VAS assessments and shown an example. Owners were then asked “Please indicate the extent to which your dog scratches its shoulder, neck or face:” upon a 100mm line between “Never” on the left and “Very frequently” on the right. The position
marked by the owner was measured in millimetres from the leftmost point and was expressed as a visual analogue score (VAS) between 0 (“Never”) and 100 (“Very frequently”).

2.3. Eye temperature recording

Eye temperature was recorded by taking a thermal image of the dog at an emissivity of 0.96 from 50 centimetres away. An audible toy was used to attract the dog’s attention to the camera. When the dog was standing straight, facing the camera and in focus a thermal image was taken and maximum temperature of the eye found using ThermaCAM reporter 2000 Professional software.

2.4. Judgement bias test

To measure cognitive bias the equipment was assembled as in Fig. 1. Five pre-determined locations, 4m in front of the dog’s fixed starting location were marked on the floor, or the maximum possible arena size in smaller rooms. The baited positive (P) and un-baited negative (N) location were randomly assigned such that P could be on the left or right of the dog and N in the other position. The methodology was identical to that reported by Mendl et al. (2010b). Dogs were held behind a barrier by an experimenter (AD) while a food bowl was baited with three small pieces of food (Cheddar cheese), or not baited. The bowl was placed at N (if not baited) or P (if baited) and the barrier lifted to release the dog (Fig. 1). The latency to reach the bowl was recorded (capped at 30 seconds).

During the training phase, the first four trials were 2 positive (P) followed by 2 negative (N) trials. If the dog did not approach the bowl within 30s, the experimenter tapped the side of the bowl to encourage the dog to approach. Following this, negative and positive trials were presented in a pseudorandom order, with no more than three trials of the same type presented consecutively. The learning criterion was reached when for the preceding 5 positive and 5 negative trials the dog was
always quicker to P than N. If the dog had not reached the learning criterion within 50 trials, the dog did not progress to the next phase of the study.

During the testing phase, dogs were presented with non-reinforced probe trials, performed identically to the training trials but with unbaited bowls placed in one of three intermediate ambiguous locations; near positive (NP), middle (MID), or near negative (NN) (see Fig. 1). Each testing location (NP, MID and NN) was presented twice (6 probe trials in total), ordered pseudo-randomly such that all 3 testing locations were presented during both the first 3 probe trials and the last 3 probe trials, and interspersed with 2 or 4 trials with a baited bowl placed at P or an unbaited bowl at N. Following the 6 probe trials, a baited bowl was presented at the negative location as a ‘false negative’ and the latency recorded, to test if dogs were using olfactory cues to indicate reward location.

2.5. Sensitivity to reward loss test

Using the same arena setup as the cognitive bias task, the bowl at P was then baited with a single small piece of food that was one quarter of the size of the initial pieces used (i.e. one twelfth of the initial quantity). Twelve consecutive trials were run to location P as previously, and trials were stopped if the dog did not go to the bowl on 3 successive trials. Following the test, a final trial was carried out in which an unbaited ‘false positive’ bowl was presented at the positive location, in order to assess whether the dog was using olfactory cues to discriminate between baited and unbaited bowls. This was done by comparing the latency to the unbaited bowl with the average latency to the baited bowl at P during the judgement bias task.

2.6. Jump up/jump down test
To record jump-up latency, a 60cm (length) x 60cm (width) x 30cm (height) pouffe footstool was placed on the floor 1m away from the dog. A piece of food was dropped into a bowl on top of the footstool and the dog was then released to allow it to jump up on to the stool and consume the food. The dog was allowed a maximum of 20 seconds to retrieve the food, and its latency to do so was recorded. An average latency was calculated over 3 repeats of this test. The technique was then repeated to assess jump-down latency. The dog placed on the footstool and the baited bowl on the floor 1m away, and the latency for the dog to jump down from the footstool to reach the bowl was recorded over 3 repeats of the test.

2.7. Data preparation and statistical analysis

For the judgement bias test, latencies (seconds) to each probe location were calculated and averaged across the two repeats per location. Mean latencies to the P and N locations were calculated from the 3 trials preceding, and all trials during, the testing phase. Dogs had different baseline running speeds to the positive and negative bowl and some arena sizes were slightly smaller than standard (4m by 3m) due to limitations of testing in the owner’s home. To control for this when comparing SM and SM-free dogs, an adjusted latency ($i_a$) was calculated to give a score for each probe (ambiguous) trial relative to each dog’s average speed to the baited (P) and unbaited (N) bowls, using the formula:

$$i_a = \frac{(i-p)/(n-p)}{100}$$

Where ‘p’ is the mean latency to the positive bowl, ‘n’ is mean latency to the negative bowl and ‘i’ the absolute latency to the intermediate bowl on that trial.

During the reward-loss sensitivity task, for trials on which the bowl was not visited and trials that were stopped before 12 trials were complete, the latency to reach each bowl was coded as 30s (the
maximum time given to dogs to approach the bowl. Each dog’s mean latency to the P location, calculated as described above, was subtracted from its latency to the bowl on each trial as a measure of the increase in approach latency relative to baseline.

Data were analysed using IBM SPSS Version 23. For each dataset, relevant assumptions for parametric tests were checked including, as appropriate, Shapiro-Wilk tests of normality, Levene’s tests of homogeneity of variance and Mauchly’s tests of sphericity. Where assumptions were not met, logarithmic transformations of the data were initially performed. If these were unsuccessful (e.g. for unadjusted latencies to approach the bowl in the judgement bias task; latency data in the reward-loss sensitivity task), nonparametric alternatives (e.g. Friedman test, Mann-Whitney U test, Spearman rank correlation) were used. Other details of statistical tests are given with their relevant results.

3. Results

3.1. Signalment, scratching score, and arena size

21 dogs (78%) completed the judgement bias task (8 dogs with SM and 13 without). Fourteen dogs were female (67%; 5 with SM and 9 without SM) and seven dogs were male (33%; 3 with SM and 4 without SM). The mean age of dogs that completed the judgement bias task was 65 ± 9.5 months (5.4 ± 0.8 years). There was no difference in age between dogs with SM (median 69 (IQR 45-80.25) months) and dogs without SM (median 51 (IQR37-76.5); Mann-Whitney U test U=38.5, z=-0.978, p=0.336). Owners reported significantly higher scratching scores in SM dogs than in SM-free dogs (U=23, z=-2.161, p=0.037; Fig. 2).

Arena sizes varied between owner homes but there was no significant correlation between the area of the arena and the mean adjusted latency to all 3 probe locations ($Q=0.107$, $n=21$, $p=0.645$) or the
mean increase in latency across all trials during the reward loss sensitivity task ($\rho=0.158$, $n=21$, $p=0.495$). Furthermore, there was no significant difference between the areas of arenas used for dogs with and without SM ($U = 41.5$, $z=-0.786$, $p=0.456$).

3.2. Judgement bias test

All dogs reached criterion during the training phase of the judgement bias task in a median of 21 trials (IQR 18.5-27), and SM diagnosis did not affect learning speed (SM: median 25 (IQR 19.25-29.5); SM-free: median 21 (IQR 17-22.5); $U=34.5$, $z=-1.274$, $p=0.21$). In the testing phase, unadjusted latency data ($n=21$; SM and SM-free dogs pooled) were used in a within-subjects analysis to investigate whether dogs discriminated between P and N locations and how this generalised across ambiguous locations. Bowl location affected latency (Friedman’s test: $\chi^2=47.42$, $n=21$, $p<0.001$), with dogs reaching the P location fastest, N location slowest, and showing intermediate latencies to the NP, MID and NN locations, indicating that they had learnt the task (Fig. 3).

To compare responses of SM and SM-free dogs to the ambiguous probe locations (Bateson & Nettle 2015; Bateson et al. 2015), adjusted latency data were used to control for differences in individual running speed and arena size. A mixed model ANOVA was constructed with adjusted latency as the dependent variable, SM (presence/absence) and medication (medicated/unmedicated) as between-subject variables, ambiguous bowl location (near positive, middle and near negative) as a within-subjects variable, and scratching VAS score as a continuous covariate. Medication was then removed from the initial model as it had no significant effect ($F_{1,16}=2.520$, $p=0.132$), and the model was recalculated using the remaining factors.

There was no significant effect of location on adjusted latency to reach the bowl ($F_{2,34}=1.395$, $p=0.262$) and no significant interactions with bowl location ($p>0.05$). There were significant effects of SM diagnosis ($F_{1,17}=5.201$, $p=0.036$) and scratching score ($F_{1,17}=6.098$, $p=0.02$) with SM dogs (Fig. 4a).
and dogs who scratched less (Fig. 4b) being slower to move to the ambiguous locations. Fig 4b
indicates that this latter relationship was stronger in SM dogs than SM-free dogs, although this was
not significant (scratching score * SM diagnosis interaction (F_{1,17}=1.107, p=0.307)).

3.3. Reward loss sensitivity test

Food reward was reduced from 3 pieces of cheese to 0.25 pieces on trial 1, and for all subsequent
trials. Testing was stopped for four dogs (2 with SM: stopped after trials 9,11; 2 without SM: stopped
after trials 6,10) who failed to visit the bowl on 3 consecutive trials. There was no difference
between SM diagnosis groups in the number of trials completed (U= 48, z=-0.422, p=0.804). Latency
to approach the bowl relative to baseline (mean latency to the P location) was strongly affected by
trial (Friedman test: X^2=79.42, n=21, p<0.001) indicating a decrease in speed to move to the bowl
across trials, especially between the first 3 and later trials (Fig. 5). To minimise multiple comparisons
of the effects of diagnosis on relative increase in latency to the bowl, data for each individual were
averaged across blocks of trials (1-3, 4-6, 7-9, 10-12). There were no significant differences in
increase in latency between SM and SM-free dogs, or between medicated and non-medicated dogs,
during any trial block (Mann-Whitney U-tests, p>0.05 for all).

3.4. Tests of the use of olfaction to detect the food reward

There was no significant difference between latencies to reach the positive and false positive bowls
(Wilcoxon test Z=-0.608, n=21, p=0.543). The latency to reach the false negative bowl (median 30
(IQR 20.05-30)) was actually greater than that to reach the negative bowl (median 20.65 (IQR 15.13-
27.81), Z=-2.133, n=21, p=0.033), indicating that dogs were not using olfactory stimuli to detect and
preferentially approach when food was present.
3.5. Syrinx size

For dogs with SM, mean syrinx size was 4.20 ± 0.97mm (n=5; exact syrinx size was unknown for three dogs diagnosed with SM following MRI). Syrinx size was not significantly correlated with VAS scratching score ($\varrho=-0.5$, N=5, p=0.391) or with the mean adjusted latency to all 3 probe locations in the judgement bias task ($\varrho=-3.59$, N=5, p=0.553). Dogs without SM all had a syrinx size of 0mm and were not included in these analyses.

3.6. Eye temperature

A t-test revealed no significant difference in eye temperature ($t(19) = 0.122$, p=0.904) between dogs with SM (34.69±0.262°C) and dogs without SM (34.73±0.256°C). Furthermore, there was no significant correlation between eye temperature and mean adjusted latency to all 3 probe locations in the judgement bias task ($r=-0.118$, N=21, p=0.611).

3.7. Jump up/jump down test

There were no significant differences between diagnosis groups in the mean latencies (s) to jump up onto or down off the footstool (jump up: SM median 1.84 (IQR 2.41-7.75); SM-free median 4.17 (IQR 2.62-7.7); U=39.0, z=-4.13, p=0.717; jump down: SM median 3.87 (IQR 1.3-2.5); SM-free median 1.98 (IQR 1.78-3.23); U=32.0, z=-0.991, p=0.322). Age correlated positively with both latency to jump up ($\varrho=0.505$, N=19, p=0.027) and latency to jump down ($\varrho=0.486$, N=19, p=0.035). Both latencies were strongly positively correlated with each other ($\varrho=0.767$, N=19, p<0.001).

4. Discussion
Dogs achieved performance criterion on the judgement bias task in a median of 21 trials. A within-dog analysis of unadjusted running speed showed that they successfully discriminated the P and N locations, and responded to the intermediate locations as predicted if making a spatial generalisation of the location-reward contingency. To compare how dogs with syringomyelia (SM) and SM-free dogs responded to the ambiguous locations, latencies were adjusted to account for the effects of individual differences and varying test arena sizes on baseline running speed to P and N locations. Dogs with syringomyelia were significantly slower relative to their baseline running speed to reach the ambiguous bowls than SM-free dogs, indicating a relatively negative judgement of ambiguous stimuli. This is in line with other studies demonstrating that putative negatively valenced affective states, including pain, induce negative judgement biases (e.g. Harding et al., 2004, Burman et al., 2008a, Mendl et al., 2010b, Neave et al., 2013). Olfactory detection of food rewards was unlikely to have influenced these results because, in tests of this possibility, dogs ran just as fast to an unbaited bowl in the positive location as they did to a standard baited bowl in this location, and actually ran more slowly to a baited bowl in the negative location than to a standard unbaited bowl in this location. This latter result may have occurred because the ‘false negative’ trials were performed some time after those used to calculate mean running time to the N location, and hence dogs would have had further time to learn that bowls in the negative location did not contain food. Dogs with syringomyelia had higher owner-reported scratching VAS scores than SM-free dogs, in line with the finding that scratching is a commonly-reported sign of SM (Plessas et al., 2012). It was hypothesised that increased scratching would indicate increased severity of SM, and thus that it would correlate negatively with affective state, and hence be associated with increased latency to reach the ambiguous bowl locations. However, the results of this study show that higher scratching VAS scores were associated with shorter rather than longer latencies to approach ambiguous bowl locations, suggestive of a relatively positive affective state. One possible explanation is that scratching functions to relieve discomfort caused by SM, as is known to occur with acute itch (Davidson et al., 2009), and hence decreased distress in this way.
Another possibility is that much of the scratching reported by owners was phantom scratching and that this is not directly related to pain. Phantom scratching has been shown to be associated with MRI findings of a large syrinx extending into the mid cervical superficial dorsal horn. The action is very similar to fictive scratching which occurs in animals with severed spinal cords (Sherrington 1906) and it is hypothesised that it is not a behavioural response to a perceived discomfort but due to damage to a population of spinal cells which influence the lumbosacral central pattern generator (Nalborcyk et al. 2017). The possibility that the VAS scratching score primarily reflected phantom scratching could explain why dogs who had a higher score did not also show a negative judgement bias, but not why they showed a more positive judgement bias. Since the scratching score used in this study did not allow discrimination between phantom scratching and scratching in which the dog makes contact with the body, it was not possible to investigate this possibility further.

In the reward loss sensitivity test, dogs increased their latency to move to the positive bowl location after the available reward had been decreased to a quarter of its previous size. In the absence of an appropriate control group, it is not possible to determine whether they also showed a successive negative contrast (SNC) effect and slowed their responding in comparison to those who had always been presented with the smaller reward size. Bentosela et al. (2009) observed such an effect, but Reimer et al. (2016) failed to replicate it. However, the aim here was to investigate whether SM dogs showed a stronger response to reward loss than SM-free dogs, as observed in depressed compared to non-depressed humans (Chiu and Deldin, 2007, Hajcak et al., 2004) and rats in unenriched compared to enriched housing (Burman et al., 2008b). There was no evidence of this effect. As concluded by Reimer et al. (2016), further work is required to determine whether this type of test can: (i) generate SNC effects; (ii) identify whether dogs in putatively different affective states show different sized SNC effects, including when used under non-laboratory conditions.

There were no significant differences in eye temperature between dogs with and without SM, and no correlation between eye temperature and judgement bias scores. This is possibly because studies...
that have detected a pain-related increase in eye temperature have done so following acute or evoked pain (Stewart et al., 2008, Stubsjøen et al., 2009, Cook et al., 2006). Dogs with SM are thought to have chronic neuropathic pain (Plessas et al., 2012), which may not cause increases in eye temperature in the same way. Additionally, CKCSs are predisposed to a wide range of ophthalmic disorders (Belknap et al., 2015), and thus their ocular blood flow and eye conformation may differ anatomically and physiologically to that of the wild-type ancestors of dogs, potentially affecting their eye temperature variation in response to pain. Therefore, the use of eye temperature measures as indicators of pain may have limitations in this breed.

There was also no difference in latency to jump up or down from a footstool between dogs with and without syringomyelia. It thus appears that the dogs recruited for this study did not have significantly impaired mobility, or a lowered threshold of movement-induced pain (as measured by reluctance to jump), due to their syringomyelia. Although older dogs did have increased latencies to jump up or down, there was no significant difference in the ages of dogs with and without syringomyelia in this study. Older dogs are more likely to have osteoarthritis (Henrotin et al., 2005), which is known to impair mobility (Wernham et al., 2011). Dogs diagnosed with painful conditions like this were excluded from the study, but it is possible that undiagnosed osteoarthritis may have been present, and may have caused the increase in latency to jump up and down in older dogs.

Most dogs with SM (and some found not to have SM following MRI) in this study were on medication. This included drugs to reduce CSF pressure and thus treat SM directly (omeprazole and cimetidine). In addition, some dogs were receiving medication to treat pain associated with the condition such as nonsteroidal analgesics (mavacoxib, carprofen), corticosteroids, opioid analgesics, and gabapentinoid analgesics (gabapentin, pregabalin). Many dogs were on various combinations of these drugs so it was not possible to assess their effects individually, and furthermore it was not possible to withhold medication during the study for ethical reasons. However, the presence or absence of medication did not have any effect on latency to approach any of the ambiguous bowls
or on reward-loss sensitivity. This suggests that the differences seen between SM and SM-free dogs in this study were not caused by medication, and implies that they occurred as a result of syringomyelia itself. Our findings might also indicate that, even with medication, some SM dogs may have been experiencing a negative affective state, implying that medication may not always fully control the effects of syringomyelia. This is in line with the findings of Plessas et al. (2012) who observed that clinical signs of 75% of dogs diagnosed with syringomyelia continued to worsen following diagnosis and medical treatment, such that 14.6% were euthanased prior to study completion due to clinical signs of neuropathic pain. It thus appears there is an unmet need for more effective treatments of syringomyelia in CKCSs.

Whilst it is possible that the negative affective state implied by the negative judgement bias observed in this study was due to neuropathic pain caused by syringomyelia (Plessas et al., 2012), it could also be due to other clinical signs associated with the disease. In humans, whilst 50-90% of syringomyelia patients report pain, many also report a sensation of burning, tingling or stretching of the skin (Todor et al., 2000) which could cause discomfort in dogs too. Human patients sometimes experience impaired proprioception (Masur et al., 1992) which, if present in dogs, may cause negative affective states by interfering with perceived behavioural control. People with Chiari malformation have a high incidence of sleep apnoea (Gagnadoux et al., 2006) that causes restless sleep and decreased quality of life (McArdle et al., 2001). If this occurs in dogs, it may induce similar negative states. Therefore, whilst pain is a very common feature of syringomyelia in humans (Milhorat et al., 1996) that causes a negative emotional state (Hummel et al., 2008, Gaskin et al., 1992), there are other seemingly unpleasant features of human syringomyelia that, if also present in dogs, may cause or contribute to negative affect.

5. Conclusion
This study provides a first indication that CKCSs with syringomyelia display a relatively negative judgement of ambiguity compared to SM-free dogs, suggesting that syringomyelia induces a negative affective state. Further confirmation of these results is required in studies with larger sample sizes that may be able to address some of the alternative explanations listed above and, importantly, allow the effects of medication to be more carefully analysed. Should such studies generate similar findings, they will indicate that changes to breeding and showing practices could allow selection to decrease the risk of syringomyelia and the negative affective states that may accompany it.
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Figure legends

Figure 1: Plan view of the set-up for Judgement Bias testing, including the two training locations, P and N, and the three ambiguous locations, NP, MID and NN. The standard arena length between start point and bowl location was 4m, with 3m between the P and N locations. However, some arenas were smaller than this.

Figure 2: Scratching scores of dogs with and without SM. Box-plots show medians, quartiles and ranges. Data points are indicated if they are greater than 1.5 (circle) or 3 (asterisk) inter-quartile ranges away from the upper or lower quartile.

Figure 3: Median unadjusted latency to reach each bowl location in the judgement bias test. Box-plots show medians, quartiles and ranges. Data points are indicated if they are greater than 1.5 (circle) inter-quartile ranges away from the upper or lower quartile. Pairwise Dunn-Bonferroni test significant differences (p<0.005 for all) were found between locations that do not share any of the same letter superscripts.

Figure 4: (a) Mean (+/- sem) adjusted latency to reach ambiguous bowls in SM and non-SM dogs. Data from all three ambiguous bowls are pooled as no significant effect of bowl location was found. (b) Mean adjusted latency to reach ambiguous bowls against scratching score for each dog. SM dogs are shown as filled circles, and SM-free dogs as open circles. Lines represent linear regression functions for each diagnosis, defined as $y=85.43-0.76x$ ($R^2= 0.471$) for dogs with SM (solid line) and $y=37.49-0.31x$ ($R^2= 0.116$) for dogs without SM (dashed line).

Figure 5: Increase in latency to move to food bowl relative to baseline across all 12 trials of the Reward loss sensitivity test. Box-plots show medians, quartiles and ranges. Data points are indicated if they are greater than 1.5 (circle) or 3 (asterisk) inter-quartile ranges away from the upper or lower quartile. Pairwise Dunn-Bonferroni test significant differences (p<0.05 for all) were found between trials that do not share any of the same letter superscripts.