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Small Animal Internal Medicine Neurology

Nasal Stimulation Response in Neurologically Normal Dogs and Cats

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ABSTRACT

Background: The range of normal responses to nasal stimulation (NSR) observed in neurologically intact dogs and cats has not been reported.

Hypothesis/Objectives: Report the responses range while performing NSR in neurologically normal dogs and cats.

Animals: Twenty dogs and twenty cats, all neurologically normal.

Methods: Prospective descriptive study. Nasal stimulation response testing was performed four times in each animal. Responses to NSR were recorded and described. Neuroanatomical cadaveric evaluation was then performed to attempt to correlate the responses with anatomy.

Results: Withdrawal of the head was the most common response observed in both dogs and cats; it was present in all animals and during 150/160 stimulations. Other observed responses included lip lick response (39/40 animals and in 121/160 stimulations) and startle response (17/40 animals and in 34/160 stimulations). The latter was present in cats significantly ($p = 0.026$) more often (8/80 of stimulations in dogs and in 26/80 of stimulations in cats). The location of the examination (home vs veterinary hospital), stress, or covering the eyes did not significantly affect the responses.

Conclusion and Clinical Importance Relevance: Withdrawal of the head is a consistent response, but in its absence, looking for lip lick or a startle response could support the normal function of the pathway.

1 | Introduction

The nasal stimulation response (NSR) test is part of the small and large animal neurological examination and aims to assess the conscious perception of nasal stimulation. The test allows both evaluation of the sensory innervation to the nares and observation of the behavioral response to the stimulation.

The nasal mucosa of the nostrils is innervated by sensory terminal branches of the ethmoidal nerve [1]. This nerve is a branch of the nasociliary nerve that has re-entered the cranial cavity through the ethmoidal foramen of the orbit, crossed the cribriform plate of the ethmoidal bone, and distributed through the nasal septum, dorsal portion of the nasal cavity, nasal conchae, and up to the mucosa and skin of the nostrils [1]. Sensory receptors on

Abbreviations: FAS, fear, anxiety and stress; HR, heart rate; NSR, nasal stimulation response.

the nasal mucosa are stimulated following touch/pressure with a small blunt instrument (forceps or cotton tip applicator) [2]. The general somatic afferent axons course from the receptor organ to the trigeminal ganglion before entering the pons [3]. First-order neurons reach the ipsilateral pontine sensory nucleus of the trigeminal nerve and the spinal tract of the trigeminal nerve before reaching the nucleus of the spinal tract of the trigeminal nerve. Second-order neurons from these nuclei cross the midline and ascend to the ventrocaudal nucleus of the thalamus as the trigeminal lemniscus, forming part of the medial lemniscus. From there they will project to the contralateral somatosensory cerebral cortex [4] (Figure 1). Stimulation of the nasal mucosa leads to a behavioral response elicited by the contralateral forebrain [2]. It is generally accepted that the normal response is a withdrawal of the head. Note that if the clinician knows that the animal has sensation in its head and face based on normal brainstem reflexes, then the absence of a behavioral response can indicate a failure to integrate sensory information at the cortical level due to a lesion in the contralateral hemisphere.

There are no studies evaluating the NSR in normal dogs or cats. The authors have anecdotally noticed differences in the response and behavior between these two species. We hypothesize that cats respond differently to NSR compared to dogs, and that in addition to pulling the head away, nasal stimulation frequently induces a lip lick response. The aims of this study were to (1) evaluate and describe the response to nasal stimulation in neurologically normal dogs and cats, (2) review and critically assess the neuroanatomy and the most rostral segment of NSR pathways in cadaveric specimens.

2 | Material and Methods

This prospective descriptive study was approved by the School Research Ethics Committee of the University of Glasgow (reference number EA27/21). An informed client consent was obtained prior to examination in all dogs and cats enrolled. Twenty cats and twenty dogs were recruited. Animals were recruited verbally by talking to friends and family. Inclusion criteria were: (1) no history of illness manifesting at the time of the examination; (2) no history of neurological disease or chronic illness; (3) age 1–10 years; (4) not receiving any medical treatment that could affect the examination. Age, breed, and sex were recorded.

Examinations were performed by a board-certified veterinary neurologist (JB) or a neurology resident in training (TE). All examinations were performed in a quiet environment with no other animals present, either at the referral hospital or at the animal's home.

The NSR is performed as part of a complete neurological examination. In order to ensure the NSR was not the only test, we also initially assessed mentation, posture and gait, proprioceptive placing in all four limbs by means of hopping (cats) and paw replacement and hopping (dogs), and menace response followed by palpebral reflex. Animals with neurological deficits at the time of the examination were excluded. The NSR was performed bilaterally twice in each patient to assess the consistency of the response. After stimulating both sides, the NSR test was repeated, starting on the opposite side. Before stimulating the opposite side, contact with the animal's head and neck was released, so the animal could look around and settle.

The NSR was evaluated by touching the mucosa of the nasal septum with the blunt end of a closed standard bent size forceps, while covering the animal's eyes (when possible) to prevent visual input. The pet's eyes were covered by the person performing the NSR test, while the forceps were directed with the opposite hand. Covering the eyes was not a mandatory requirement for

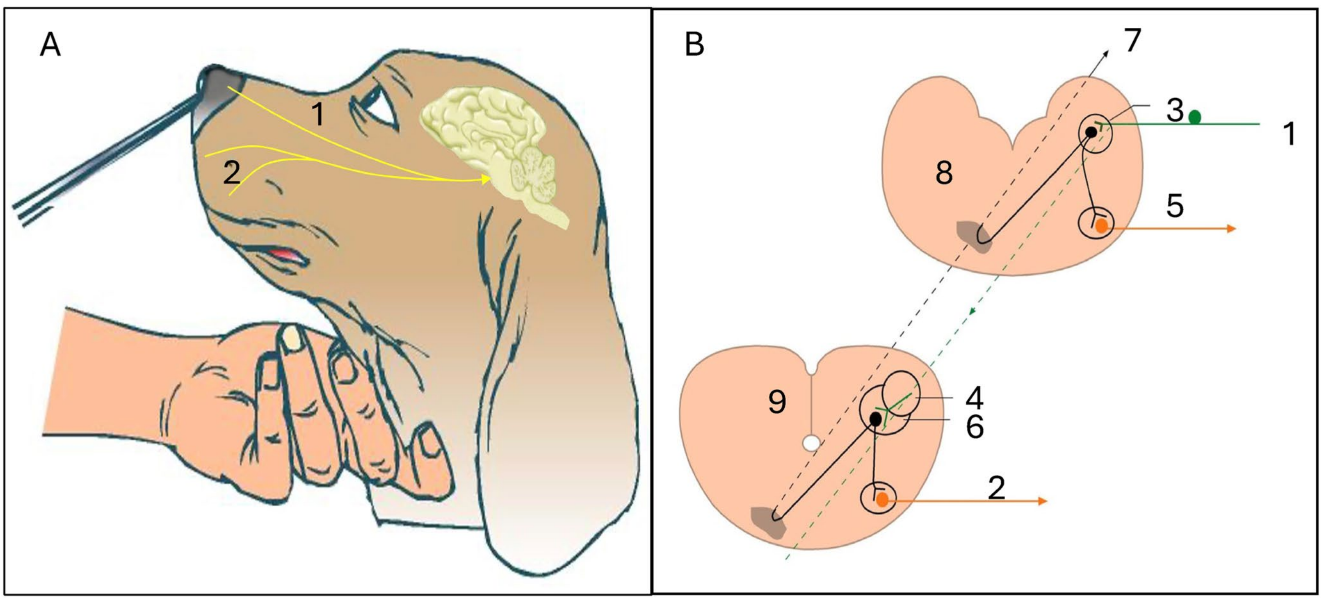

FIGURE 1 | Drawing representing the nasal stimulation response (NSR) evaluating the ethmoidal nerve (A): 1, Ethmoidal nerve. 2, Maxillary nerve. Drawing representing the trigeminal system (B): 1, Trigeminal nerve. 2, Efferent cranial nerves. 3, Sensory nucleus of the trigeminal nerve. 4, Spinal tract of the trigeminal nerve. 5, Motor nucleus of the trigeminal nerve. 6, Nucleus of the spinal tract of the trigeminal nerve. 7, Trigeminal lemniscus (medial lemniscus). 8, Caudal portion of the pons. 9, Medulla oblongata.

inclusion, given this can sometimes be difficult in nervous pets. Video footage of all NSRs was obtained by another observer positioned in front of the patient. The person performing the NSR was behind the patient and facing the person who obtained the video footage. The forceps were cleaned with water and soap in between patients. To prevent salivation and licking not associated with the NSR, no food or treats were given 30 min prior to or during assessment.

Video footage was reviewed by TE and whether the patient withdrew the head in any direction; had a lip lick response; a startle response; or a combination of the above was recorded. For a positive lip lick response, the patient had to lick its lips within 5 s of the NSR test. Lip lick response was classified as immediate (if the animals licked their lips immediately after the NSR test), delayed (if they licked their lips two-five seconds after the NSR test) or absent if they did not lick their lips or if they did so more than 5 s after the NSR test. Startle response was considered present if the animal showed a rapid extension and flexion of the head and/or neck muscles in response to the tactile stimulus caused by the NSR test.

Whether the examination was performed at home or at the veterinary hospital, whether the patient was stressed during the examination, and whether the eyes were covered while testing the NSR were also recorded. Animals were considered stressed if they appeared unsettled or were wriggly while the examination was performed. A cadaveric study of the nasal cavity and evaluation of the terminal branches of the trigeminal nerve ramifications within the nasal cavity were performed in one dog with no history of neurological disease. The anatomic dissection was performed (VA) in a canine cadaver from the Anatomy Unit of the Veterinary Faculty donated by the owners following the approved program of the Universidad Autónoma of Barcelona and used for teaching and research purposes. The specimen was fixed with a 10% formaldehyde buffered solution injected using the common carotid artery and preserved for 3 weeks at 4°C–6°C before dissection.

3 | Statistical Analysis

The percentage of times each response (withdrawal of the head, lip lick response or startle response) was observed was calculated across all observations. The number of times out of four observations that each animal showed a response was used as the basic unit for statistical analysis. Comparisons of dogs versus cats were made using Mann–Whitney tests adjusted for ties. For each species separately, comparisons of home versus hospital and of stressed versus not stressed were also made using Mann–Whitney tests adjusted for ties. However, in the assessment of eyes covered in cats, some animals had a mix of eyes covered and uncovered in different observations. Consequently, comparisons of covered versus uncovered were made using mixed model binary logistic regression with animal as a random effect and eyes covered/uncovered as a fixed effect. Significance was taken as $p < 0.05$. Tests for association between stress and location were done separately for dogs and cats using Fisher exact tests. Analysis was undertaken in Minitab 21 and R4.3.3.

4 | Results

4.1 | Animals

Forty animals (20 dogs and 20 cats) were included. Median age for the dogs was 4.25 years (range 1–10) and the median age for the cats was 4.05 years (range 1–10). In the dog group, there were eight males (four entire and four neutered) and 12 females (six entire and six neutered) whereas in the cat group, there were 16 males (all neutered) and four females (all neutered). Breeds for the dogs included English Setter ($n = 5$), Labrador ($n = 3$), Cockapoo ($n = 2$) and one dog from each of the following breeds: Border Terrier, Goldendoodle, Dachshund, Maltese, Bull Terrier, Springador, Whippet crossbreed, Cocker Spaniel, German Pointer, and Shih Tzu. For the cats, the breeds were Domestic Shorthaired ($n = 18$), one Persian, and one Sphynx.

4.2 | Neurological Examination

As expected from healthy animals, the tested aspects of the neurological examination were normal except for the menace response; this response was normal in 17/20 cats (85%), and it was weak in the remaining three cats. The cats with an abnormal menace response were considered visually normal.

4.3 | Nasal Stimulation Response

The NSR test was performed a total of 160 times, four times per animal (two on the right side and two on the left side). The examination was performed at the animal's home in 15/40 animals (37%; $n = 7$ 35% of dogs and $n = 8$ 40% of cats); the remainder at the referral hospital. Overall, 14/40 animals (35%; $n = 5$ 25% of dogs and $n = 9$ 45% of cats) were considered to be stressed/unsettled while the NSR test was performed. When assessing the NSR, the eyes were covered in 121/160 stimulations (76%, 80/80 100% of stimulations in dogs and 41/80 51% of stimulations in cats).

4.3.1 | Withdrawal of the Head

Withdrawal of the head was present in all animals, and it was noticed in 150/160 of the stimulations (94%; 73/80 91% of stimulations in dogs and 77/80 96% of stimulations in cats). It was noticed in all 4/4 stimulations in 31/40 animals (77%; $n = 13$ 65% of dogs and $n = 18$ 90% of cats), in 3/4 stimulations in 8/40 animals (20%; $n = 7$ 35% of dogs and $n = 1$ 5% of cats) and in 2/4 stimulations in 1/40 animals (2%; $n = 1$ 5% of cats) (Figure 2). In the cat in which withdrawal of the head was only recorded in 2/4 stimulations, the response was recorded once on each side. There was no significant difference in the rate of head withdrawal between dogs and cats ($p = 0.086$); Videos (S1 and S2).

4.3.2 | Lip Lick Response

Lip lick response was seen in at least one of the four performed nasal stimulations in 39/40 animals (97%; $n = 20$ 100% of dogs and $n = 19$ 95% of cats) and in 121/160 stimulations (76%; 59/80 74% of stimulations in dogs and in 62/80 77% of stimulations in

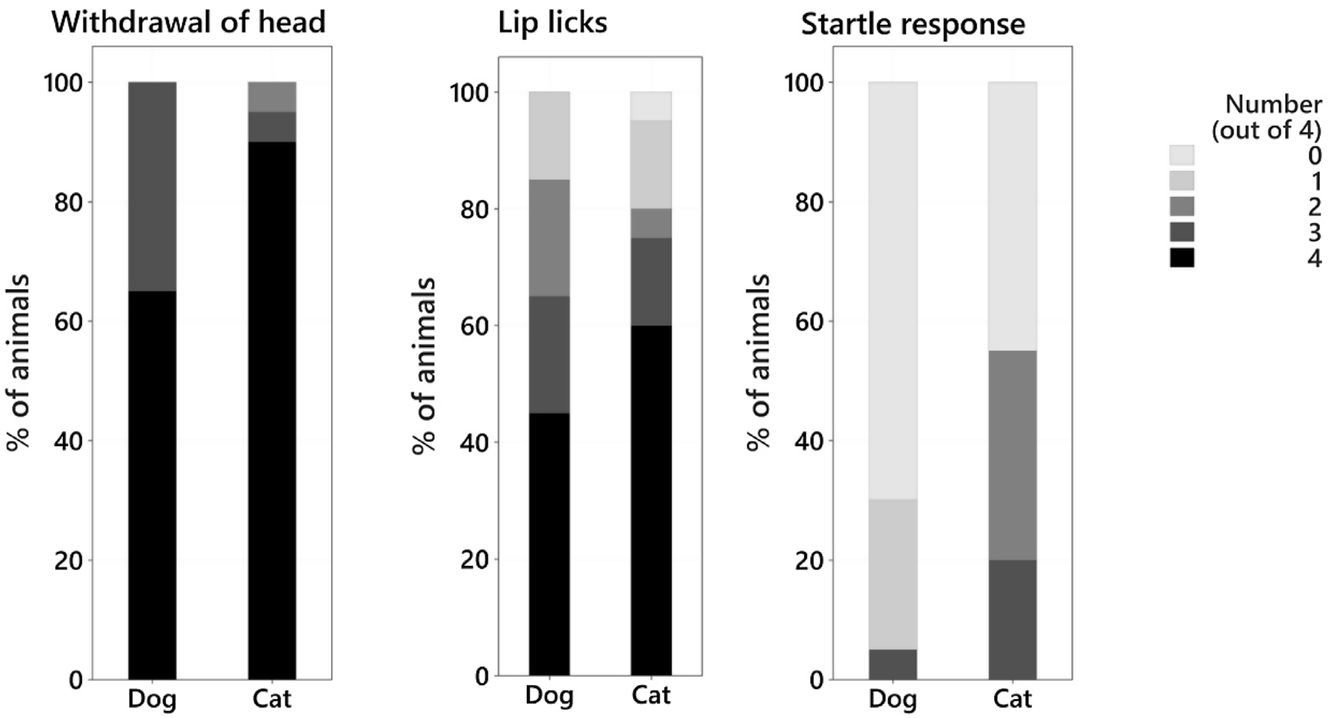

FIGURE 2 | The proportion of dogs and cats that had between 0 and 4 responses (out of 4) in the form of withdrawal of the head, lip licks, or startle response.

cats). Lip lick response was noticed in 4/4 stimulations in 21/40 animals (52%; $n=9$ 45% of dogs and $n=12$ 60% of cats), in 3/4 stimulations in 7/40 animals (17%; $n=4$ 20% of dogs and $n=3$ 15% of cats), in 2/4 stimulations in 5/40 animals (12%; and $n=4$ 20% of dogs $n=1$ 5% of cats) and in 1/4 stimulations in 6/40 animals (15%; $n=3$ 15% of dogs and $n=3$ 15% of cats); (Figure 2). There was no significant difference in the rate of lip lick between dogs and cats ($p=0.086$).

The lip lick response was immediate in 110/121 positive responses (91%; 53/59 stimulations in dogs and 57/62 stimulations in cats) and it was delayed in 11/121 positive responses (9%; 6/80 stimulations in dogs and in 5/80 stimulations in cats); Videos (S1 and S2).

4.3.3 | Startle Response

Startle response was observed in 17/40 animals (42%; $n=6$ dogs and $n=11$ cats) and 34/160 of the stimulations (21%; 8/80 10% of stimulations in dogs and in 26/80 32% of stimulations in cats). It was noticed in 3/4 stimulations in 5/40 animals (12%; $n=1$ 5% of dogs and $n=4$ 20% of cats), in 2/4 stimulations in 7/40 animals (17%; $n=7$ 35% of cats) and in 1/4 stimulations in 5/40 animals (12%; $n=5$ 25% of dogs) (Figure 2). There was a significant difference between dogs and cats in the rate of responses ($p=0.026$); Video (S2).

4.3.4 | Combinations

All three responses were seen in 17/40 animals (42%; $n=6$ 30% of dogs and $n=11$ 55% of cats). Withdrawal of the head together with lip lick was seen in 22/40 animals (55%; $n=14$ 70% of dogs and $n=8$ 40% of cats) and withdrawal of the head alone was seen in 1/40 animals (2%; $n=1$ 5% of cats). No other combinations occurred.

4.3.5 | External Influences

There were no significant differences between response rates at home or in hospital for either dogs or cats for any of the variables (head withdrawal: dogs $p=0.151$, cats $p=0.882$; lip licks: dogs $p=0.615$, cats $p=0.760$; startle response: dogs $p=0.961$, cats $p=0.454$) (Figure 3).

Likewise, there were no significant differences between response rates for stressed and unstressed animals for either dogs or cats for any of the variables (head withdrawal: dogs $p=0.460$, cats $p=1.000$; lip licks: dogs $p=1.000$, cats $p=0.197$; startle response: dogs $p=0.828$, cats $p=0.164$); (Figure 4). There was no significant association between stress and location in either dogs ($p=0.289$) or cats ($p=0.362$).

Whether covering the eyes or not had any impact on the consistency of the responses in cats was also analyzed; no significant difference was detected for any of the responses: withdrawal of the head ($p=0.453$), lip lick response ($p=0.187$) or startle response ($p=0.922$).

5 | Cadaveric Study

Inside the nasal cavity, the ethmoidal nerve (a branch of the ophthalmic nerve) divides into a medial, lateral, and external

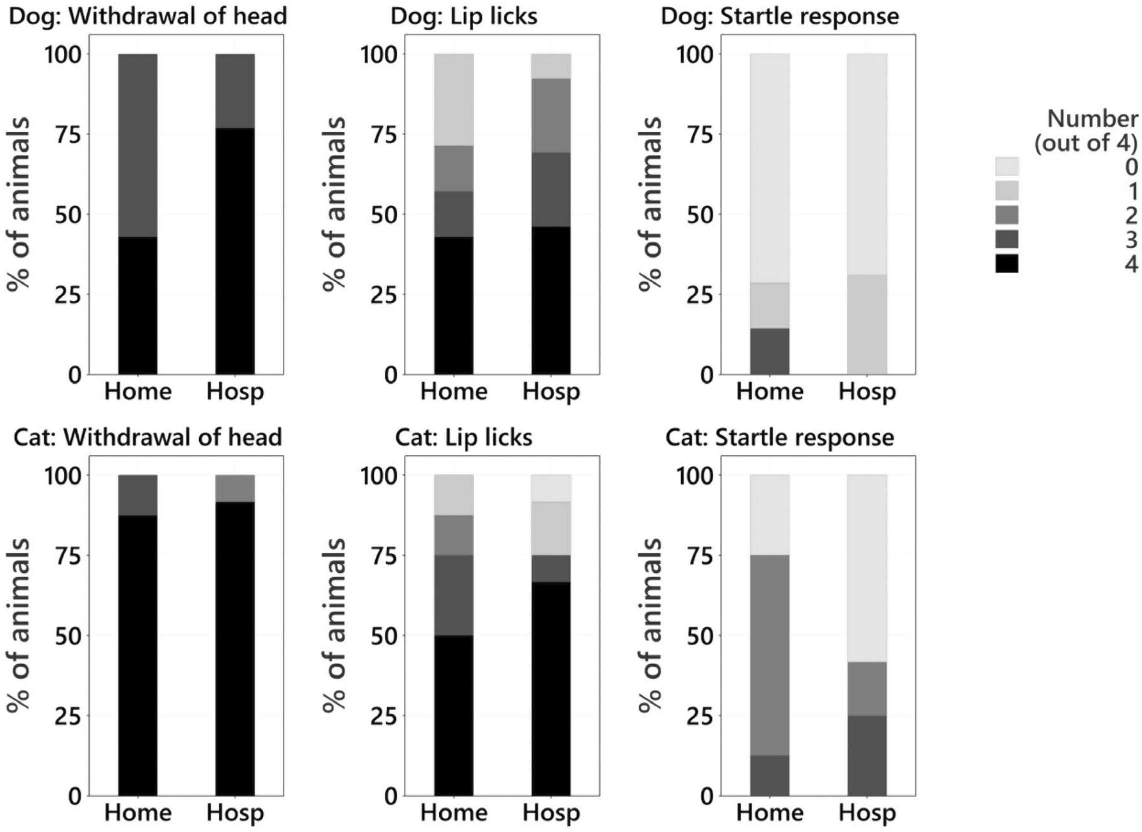

FIGURE 3 | The proportion of between 0 and 4 responses per animal (out of 4) in the form of withdrawal of the head, lip licks, or startle response in home or hospital (Hosp) locations. Dog and cat data presented separately.

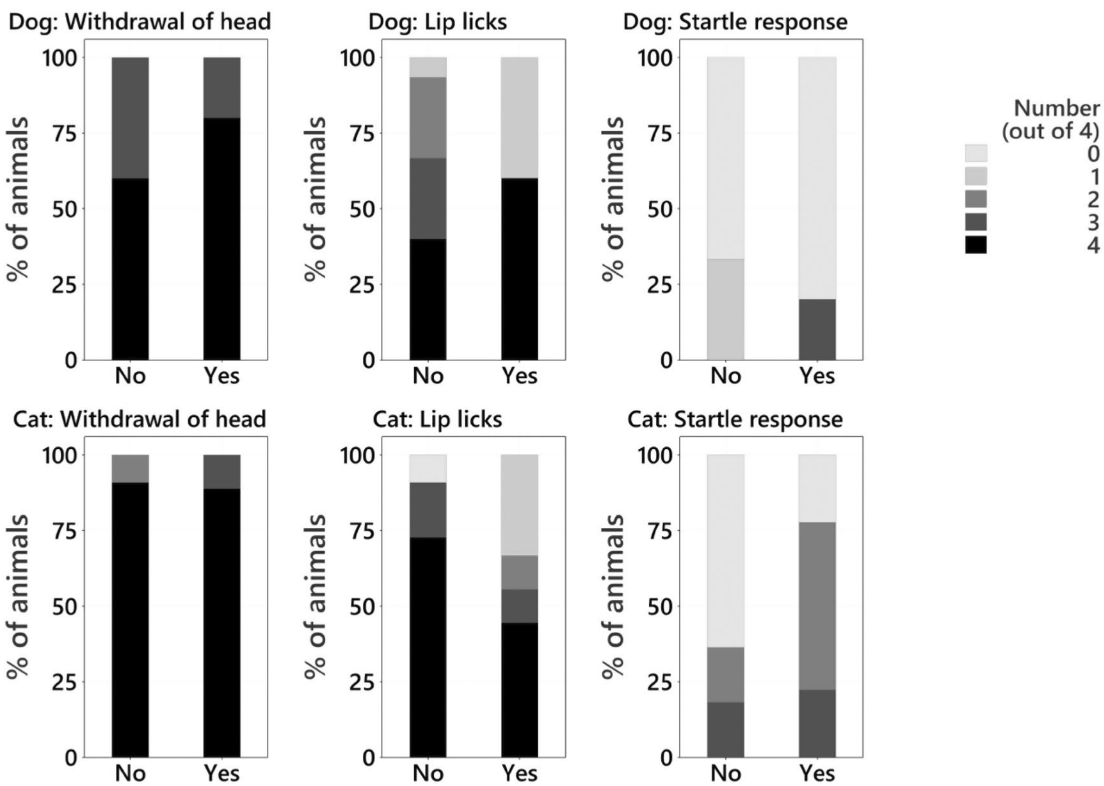

FIGURE 4 | The proportion of between 0 and 4 responses per animal (out of 4) in the form of withdrawal of the head, lip licks, or startle response in stressed (Yes) and unstressed (No) animals. Dog and cat data presented separately.

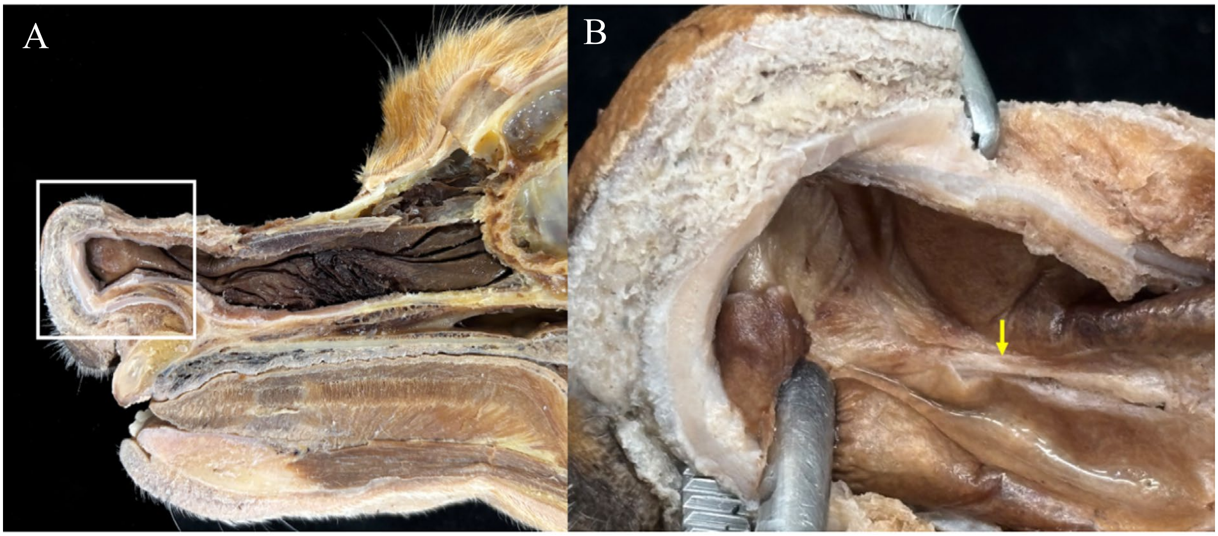

FIGURE 5 | Midplane vision of the nasal cavity of the dog (A) and close-up of the rectangle area of Figure A (B). The alar fold has been depressed ventrally and the lateral mucosa opened to identify the external branch of the ethmoidal nerve (yellow arrow) that innervates the nostrils.

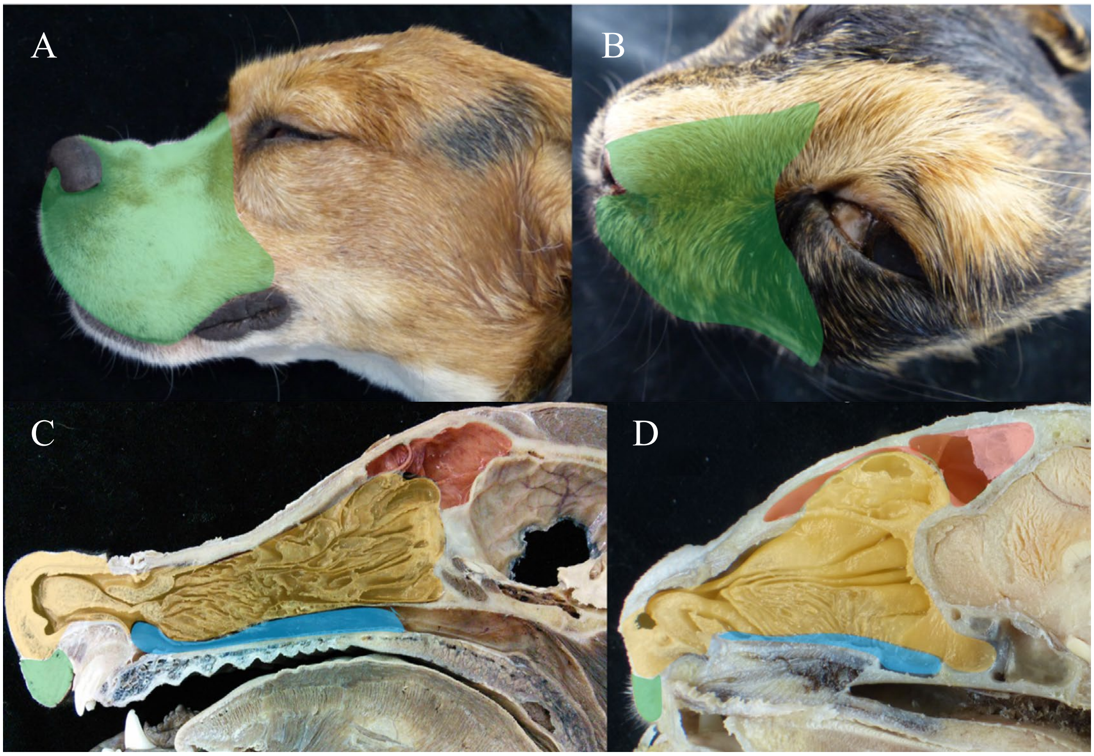

FIGURE 6 | Areas of sensory innervation of the infraorbital nerve (branch of the maxillary nerve) in green, ethmoidal nerve (branch of the ophthalmic nerve) in yellow, the caudal nasal nerve (branch of the pterygopalatine nerve) in blue, and the supraorbital and supratrochlear nerves (branches of the frontal nerve) in red of the dog (A and C) and the cat (B and D).

branches. The medial innervates the septum; the lateral innervates the nasal conchae, and the external innervates the skin of the vestibule (Figures 5 and 6). A large nerve that branches off from the infraorbital nerve (a rostral continuation of the maxillary nerve after branching off the pterygopalatine nerve) runs rostrally to sensory innervate the more rostral portion of the upper lip and the associated philtrum. From the dissections, we did not identify any branches from the infraorbital nerve to the nostrils, nor the alar region, as this area is sensory innervated by the ethmoidal nerve.

6 | Discussion

In this prospective and cadaveric study, we describe and study the NSR in dogs and cats and compare the response between these two species. Withdrawal of the head when performing the NSR is the expected response based on the current literature [2, 5, 6], and the response that neurologists typically look for. Additionally to current knowledge, we also describe lip lick and startle responses as common responses when performing the NSR test in both dogs and cats.

In our study, none of the described responses was seen in 100% of the stimulations. The most common response in both dogs and cats was withdrawal of the head, followed by lip lick response and startle response. Given that withdrawal of the head was not present in all the stimulations, repeating the test again in those animals who do not initially respond is advised. Alternatively, the clinician could also look for a lip lick response or startle response, which can be considered a positive NSR.

Despite the slightly higher rates of head withdrawal and lip lick responses in cats, there were no statistically significant differences in these responses between healthy dogs and cats. A possible explanation for the slightly higher number of cats showing a lip lick response compared to dogs might be anatomical variations in the nasal cavity, such as more sensitive receptors in the nasal mucosa, or maybe a higher concentration of sensory receptors. This could make them more susceptible to external sensory or nociceptive stimulus. On the other hand, the startled response was significantly more common in healthy cats. This finding could be explained by the different behavior between species. Dogs are usually more social than cats and more exposed to manual handling. One study assessing personalities in dogs and cats found dogs to score higher in sociability, whereas cats scored higher in neuroticism [7]. As veterinary patients are unable to verbalize feelings of stress, interpretation of stress can be challenging. In one study which evaluated the effect of clinical examination on stress in cats [8], measures of heart rate (HR) and behavioral indicators of fear, anxiety, and stress (FAS) such as eyes, vocalization, and body, tail, and ear position were used to score cat behavior during the examination at the hospital. In that study, clinically significant increases in both HR and FAS were commonly detected, particularly when cats were taken away from the owner [8]. We considered an animal to be stressed if they appeared unsettled/wriggly or if the animal was trying to escape while the examination was performed. Overall, being stressed did not significantly affect the withdrawal of the head and lick lip responses in either dogs or cats. The startle response was greater, but not significantly greater, in the apparently stressed cats. Another analysis in this study was whether the location where the examination was performed (home vs. hospital) affected the response to NSR; however, no significant differences were detected. The smaller sample size in these subset analyses would have reduced the power to detect differences.

The lip lick response seems common in both dogs and cats and it was observed in 97% of the animals and in 76% of all nasal stimulations. Licking of lips may occur in different situations, as in the context of food intake. Some pets may also lick their lips when they are stressed, anxious or in pain [9], or nauseous [10]. The animals included in this study did not have signs of gastrointestinal disease and no food was given at the time of the assessment, making these two potential influences unlikely. Stress could also cause lip lick. Animals were exposed to unusual handling; furthermore, the handling was performed by unfamiliar people. In one study performed in cats, a negative cat response to handling was evaluated [11]; the results showed that the odds of struggling were 8.2 times greater in cats being placed into full-body restraint in comparison to passively restrained cats. During the physical examination, full-body restrained cats had a faster breathing rate, more lip licks per minute, and were more likely to hold their ears in a back or side position during the first 15 s of handling, compared to passively restrained cats [11]. The lip lick response in our study was only seen immediately after or soon after performing the NSR and cats did not lick their lips otherwise. Therefore, we considered it unlikely that stress induced the lip lick response. The ethmoidal nerve innervates the nasal mucosa and constitutes the afferent limb of several upper airway protective reflexes. Protective reflexes, such as sneezing, coughing, and apnea, are those reflexes that either expel foreign substances from the respiratory tract or stop them from gaining access to the lungs [12]. There is the possibility that the NSR could have activated this mechanism, and the lip lick response might also be a reaction of protection against sternal stimulus or foreign bodies. This hypothesis was, however, considered less likely. The lick lip response is unlikely to be a reflex, since it was not consistent and in some animals there was a delay of seconds between the stimulation and the response. Overall, the most likely explanation for the lip lick response was the contact of the hemostatic forceps with the medial nasal septum and the licking may be a way of scratching the nose, or a sign of unpleasantness.

The infraorbital nerve is sensory for the skin and muscles of the lateral aspect of the nose and upper lip [13]. Given that touching the lateral part of the nare could stimulate the maxillary nerve, we aimed to direct the forceps to the nasal medial septum. It is possible that in some animals, particularly in cats due to the reduced size of the nares, the forceps touched the external part of the nare, stimulating the maxillary branch of the trigeminal nerve. Using the silicone rubber portion of a small peripheral venous catheter could have helped to stimulate the medial aspect of the nasal mucosa more accurately. However, we intentionally used a forceps in all dogs and cats as it is likely the most used tool by neurologists when eliciting the NSR.

Sensory development in both dogs and cats starts soon after birth as palpebral reflexes (CNs V and VII) develop within 2 to 4 days of birth in the puppy [14] and within 1 to 3 days in the kitten [15]. The corneal reflex in puppies (CNs V and VI) develops as soon as the eyes open (between 5 and 14 days) [14]. To the authors' knowledge, there are no references on when the NSR starts being present in puppies and kittens. The menace response is a learned response that requires all the components of the visual system pathway and connections from the cerebrum to the brainstem with activation of the facial neurons in the medulla [16]. The menace response is often absent in young animals, but it is usually present by 10 to 12 weeks of age in puppies and kittens [16]. Given that the response to nasal stimulation is also mediated by the cerebral cortex, it is possible that it is also a learned response, and it may require weeks for it to be present. Performing the NSR in newborn puppies/kittens and weekly thereafter until present would help understand this response better.

In our study, the menace response was weak in 15% of cats despite all cats considered visually healthy. The test was performed standing behind the cat, which has been reported to be the most reliable examination mode in one study assessing the menace response in neurologically and ophthalmologically healthy cats

[17]. In that study, closure of the eyelids was not complete in 15% of the cats, which matched our findings.

This study has several limitations. More than one clinician performed the examination, which could imply differences in animal handling and differences in how the NSR was performed, affecting therefore the observed response. However, the two individuals performing the observations were a boarded veterinary neurologist and a neurology resident in training under the supervision of that same clinician. Furthermore, the NSR was performed twice in each patient to assess the consistency of the response, and all the examinations were recorded and reevaluated retrospectively. Another limitation is that not all the animals were examined in the same environment (home vs referral hospital), and how stressed or relaxed the animals were could have affected responses. Pets typically undergo a neurological examination at veterinary hospitals, and therefore examining all pets at a veterinary hospital would have been preferred, but we wanted to minimize unnecessary stress for patients and facilitate normal daily routines for their owners. Examining each animal both at home and in the hospital would have been a good way to compare responses in these two different scenarios. Overall, statistical analysis failed to confirm that being stressed affected the responses. Since our ability to assess stress in animals is limited, it is possible that some animals considered stressed were not stressed and vice versa. A full physical and neurological examination was not conducted to minimize unnecessary stress on the animals. For instance, temperature and spinal reflexes were not assessed. Given our inclusion criteria and the partial physical and neurological examinations performed, it is highly improbable but not impossible that any of our study animals had neurological diseases or physical conditions that could have influenced our results. Finally, the small sample of animals hampered drawing strong conclusions.

In conclusion, we compared for the first time the responses after nasal stimulation (NSR) in dogs and cats. In addition to withdrawal of the head, lip lick response was common in both dogs and cats when performing nasal stimulation. Some animals (mostly cats) will also show a startle response. None of the described responses was seen in every stimulation, and repeating the test if absent in a dog or a cat is recommended. Alternatively, the examiner might want to look for the lip lick or a startle response. Further studies assessing the NSR in animals with trigeminal nerve lesions or structural brain disease will be necessary to evaluate whether the lip lick response is clinically useful.

Disclosure

Authors declare no off-label use of antimicrobials.

Ethics Statement

Approved by the Research Ethics Committee of the University of Glasgow (Reference EA27/21). Authors declare human ethics approval was not needed.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.