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Olfactory neuron biopsies in dogs: A feasibility pilot study☆

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Abstract

Olfactory neurons obtained through nasal biopsy were characterized. Using standard immunohistochemical techniques, nascent, developing, and mature neurons were identified in the tissues obtained. This technique shows that aspects of canine olfactory function may be potentially assessed using a relatively simple and minimally invasive biopsy technique. Such samples provide direct access to neuronal tissue that is otherwise difficult to obtain and assess in living animals. This technique may have potential value to those interested in assessing olfactory abilities in dogs.

Keywords: Nasal biopsy dogs; Canine-cognition; Olfactory neuron; Olfaction

1. Introduction

Canine olfactory capabilities are legendary (Hepper, 1988; Kalmus, 1955; Krestal et al., 1984) and exceed those of humans (Gagnon and Dore, 1992; Settle et al., 1994; Sommerville et al., 1993; Wells and Hepper, 2003). Research on detection of explosives, food, and humans has shown that olfaction provides information that allows the dog to make specific cognitive associations and then act on them (e.g., sitting next to an overseas passenger when her luggage contains a banana) (Gazit and Terkel, 2003a,b; Pickel et al., 2004; Willis et al., 2004).Interestingly, we know relatively little about the specific mechanisms of olfaction in

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dogs (Issel-Tarver and Rine, 1996; Dennis et al., 2003; Walker et al., 2006), or about olfactory neuron function and factors that could affect it (Porter et al., 2005; Shepherd, 1994, 2005). Many of the data concerning morphology and function of olfactory neurons come from mouse studies (Moberg et al., 1999). Olfactory neurons are considered to be unique in that they continuously grow throughout life, and so they have been studied for the effects of disease and aging on neuronal growth (Arnold et al., 1998, 2001; Moberg et al., 1999). Olfactory neurons lie immediately beneath the nasal epithelium, and as such, may be some of the most accessible neuronal tissue available. Biopsy is simple and virtually atraumatic in humans (Arnold et al., 1998, 2001).

This paper is a test and proof of feasibility study demonstrating that olfactory neuron morphology can be assessed in a relatively non-traumatic matter in anesthetized dogs, as it has been in humans, using local anesthesia.

2. Subjects, materials and methods

Three 4 mm samples of olfactory neuronal epithelium were obtained from the medial nares of six intact male beagle dogs ranging in age from 104 to 209 months using nasal biopsy forceps (Miltex 20-1002). Anesthesia was induced with IV diazepam, oxymorphone, and propofol, and maintained with inhaled isofluorane. Patient care before and after the procedure was routine. Biopsies were obtained after spraying each nostril with lidocaine to minimize bleeding. In this pilot study no clotting profiles were done as none of these dogs had ever experienced clotting difficulties during routine venipuncture. If clotting abnormalities were a concern a pre-procedure clotting profile may be a consideration, but it should be noted that these biopsies are generally smaller than many nicks encountered in daily life, and sampling disrupts no major vessels, as would venipuncture.

One biopsy sample from each nostril was placed in formalin (3.7%) and ethanol (70%) to control for any effects of brain lateralization and the ipsilateral pattern of olfactory innervation in canines. After fixation, these 4 mm samples were embedded in standard 10 mm paraffin blocks, and sliced at 10 μm intervals for microscopic morphological evaluation.

Samples were analyzed for characterization and morphology of neurons, and morphology of basal cells that give rise to neurons using standard published techniques (see Arnold et al., 1998, 2001 for complete details). Biopsies of olfactory epithelium (OE) were immunostained and examined using antibodies for olfactory marker protein (OMP; 1:2000), the definitive marker for mature olfactory neurons, GAP43 (1:1000) for growing immature neurons, and p75NGFR (1:10), a marker of nascent and immature neurons. Additionally biopsies were immunostained for amyloid-beta, and then counter-stained with Cresyl violet to identify neurons. These markers were chosen so that results would be comparable to those published for humans. The p75GFR antibody identifies a receptor to trophic, or growing, molecules and is expressed, selectively, in the precursor basal cells in the OE. As basal cells begin to mature expression of p75NGFR stops and GAP43 is expressed by the immature neurons. As these neurons mature, the expression of GAP43 decreases and OMP is predominantly expressed (Calof et al., 1996; Verhaagen et al., 1989). Hence, these markers provide a portrait of sequential stages in the maturation of olfactory neurons.

3. Results

Usable, non-crushed samples were obtained for only three dogs, half of those sampled. With experience, and parallel alignment of the forceps with the medial septum, samples were obtained from a uniform plane, and cut from, rather than scooped across the epithelium so that the sample quality improved. Because of the small sample size we are restricted to a preliminary description of sample morphology. We were able to retrieve specimens with

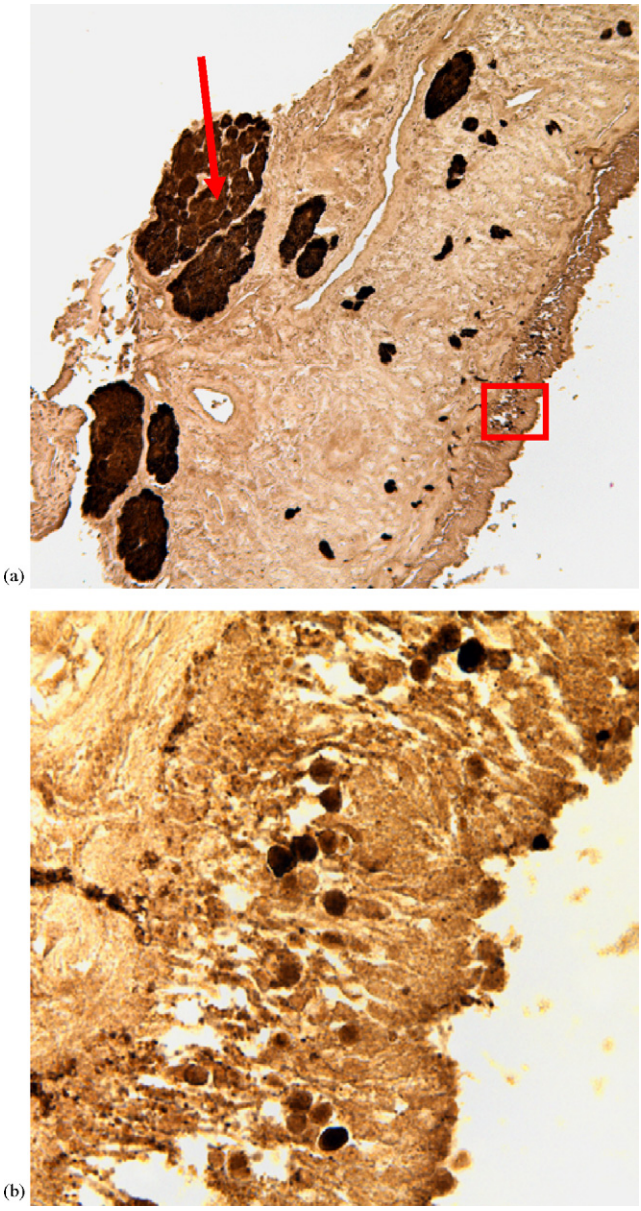

Fig. 1. (a) A sample olfactory epithelium (OE) biopsy: Olfactory marker protein (OMP) darkly stains olfactory neurons and nerve bundles (the fat, dense, regions of clumped ovals, which are neurons in cross-section; arrow is pointing to one of these). OMP is a marker of mature olfactory neurons (5× magnification). (b) 40× magnification of margin of the tissue portrayed in (a) (boxed area). Notice that at this magnification it is possible to both count the mature olfactory neurons (dark ovals and circles) and to recognize those approaching maturity.

(OMP) for all three dogs (Fig. 1), and were able to characterize developing neurons in one dog (Figs. 2 and 3). In the sample from the oldest dog we also found amyloid beta deposition surrounding and in the olfactory neurons when the sample was immunostained for amyloid beta and counter-stained with Cresyl violet to identify cells. We do not know if the presence of

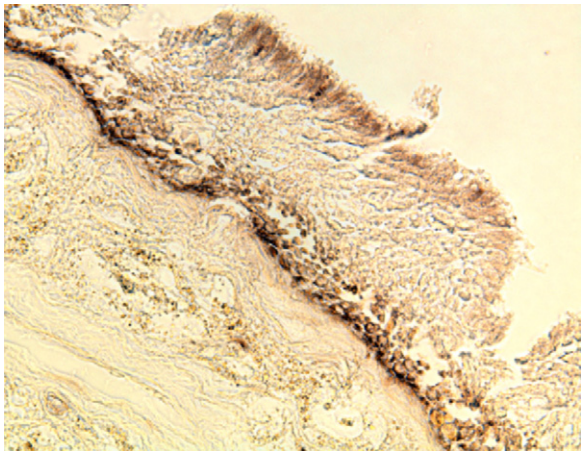

Fig. 2. Nascent and immature olfactory receptor neurons immunolabeled with the p75NGFR antibody. p75NGFR-positive cells are neuronal precursor cells, here seen as the band of dark cells adjacent to the lamina propria (5× magnification).

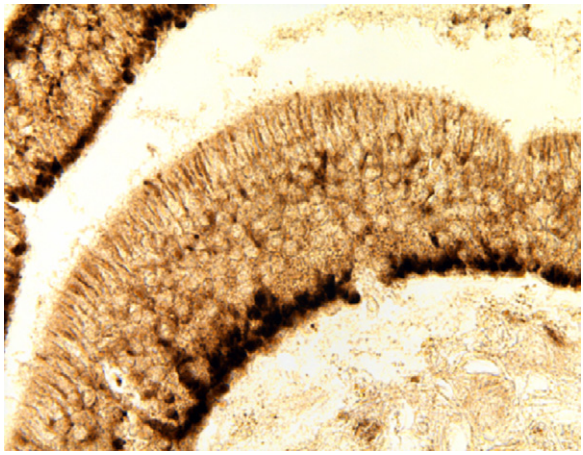

Fig. 3. Darkly stained cells are immunolabeled with the antibody for GAP43; these are growing, but still immature neurons (40×).

amyloid beta in OE correlates with that in the brain, but it is possible that OE beta amyloid may alter olfactory function.

4. Discussion

The olfactory system is viewed with increasing importance in assessments of human cognition (Arnold et al., 1998; Turetsky et al., 2000). The potential for using the olfactory system to explore related aspects of biology is only beginning to be appreciated. Olfactory neurons, because of their continuous growth and regeneration throughout life, may present a unique window into the study of cognitive changes at the cellular level that occur with age and in other neurodevelopmental

conditions. For example, in studies of human schizophrenics, densities and ratios of different maturational stages of OE neurons indicate dysregulation in this neuronal lineage when compared with matched, unaffected controls (Arnold et al., 2001). The only current data that demonstrate associations between olfactory neuronal function and cognition are from human or rodent studies (Serizawa et al., 2003). Clearly, there are opportunities for better understanding olfaction and associated cognitive aspects in dogs.

The very specific olfactory receptor genes – which are clustered at multiple loci on many chromosomes – are expressed by olfactory sensory neurons in a mutually exclusive, monoalleleic manner in the nasal olfactory epithelium (Buck and Axel, 1991; Carver et al., 1998; Issel-Tarver and Rine, 1996; Lewcock and Reed, 2004; Mombaerts, 1999; Serizawa et al., 2003). As such, olfactory neurons are the only part of the nervous system where such specific gene and receptor effects can be directly examined and potentially related to higher level function (Olender et al., 2004; Quignon et al., 2003). Our small study shows that such examinations can be done in living, functioning dogs, allowing for future studies on associations between olfactory function and cognition, including those that use repeated measures designs. Suggested future directions could include the following.

(1) Assessment of olfactory neurons may provide an early assay for conditions that affect cognition. If migration or density of olfactory neurons is altered, enhanced, or impaired, it would be logical to ask if such changes reflect neuronal patterns occurring in the brain or other regions of the nervous system. This type of approach has potential applications particularly for service and working dogs whose success may depend on specific cognitive and physical attributes. If patterns of neuronal density or turnover are reflected in performance or ability to learn, monitoring of olfactory neuronal patterns could provide insights into selection of dogs for training. This is a far reaching goal, but it may be attainable if we are interested in predictors of performance at the level of neuronal behavior, and factors that may affect it.
(2) Dogs are already used as models for human age-related cognitive change. Models for neurodevelopmental conditions using dogs could be developed, and potential links between canine olfaction and the condition of interest could be established (Head et al., 1995, 2000a,b; Milgram, 2003; Milgram et al., 1994, 1999; Siwak et al., 2000, 2001).
(3) Expansion of our pilot study could lead to an improved understanding of canine cognition and olfaction, and has the potential to provide data that will suggest how we can enhance both in working dogs. For example, we do not now know if dogs with the keenest or largest range of olfactory acuity have the same density of olfactory neurons or same proportion of nascent and developing neurons as do dogs with lesser olfactory skills. We do not know if early exposure to a variety of scents can induce olfactory neuron proliferation, and we do not know if there are preferred ages at which dogs best learn such associations. Finally, we do not know if breed affects patterns of olfactory neuron density, characterization, or migration. The current understanding of olfactory gene expression suggests that breeds tend to express the same sets of genes (Issel-Tarver and Rine, 1996; Quignon et al., 2003), so functional aspects may be very important for understanding any putative variation in breed-based olfactory capabilities. In light of the complex way canine cognition is now viewed (Hare and Tomasello, 1999; Hare et al., 2002; Kaminski et al., 2004) functional aspects of olfaction may also be complex and warrant serious investigation. Given that dogs have never been more in demand for detection purposes, consideration of these types of potential studies is particularly timely.

5. Conclusions

Three sets of our samples were not usable because the plane of the sample was not consistent and even, and the samples were crushed. The cutting surface of these forceps is so small it takes practice to remember to use them to cut and not scoop. With experience, it was possible to obtain samples from a uniform plane, at a consistent angle, and with a consistent depth, resolving this issue. However, even our small sample demonstrates that olfactory neurons can be examined in a way that may allow us to study their functional aspects in the future. Olfactory biopsy is simple and relatively atraumatic, without hemorrhage at biopsy sites during or after the procedure, and without appreciable discomfort for the dog separate from that of routine anesthesia. Olfactory biopsies can provide information about neuronal structure and function that is not otherwise obtainable *ante mortem* in dogs who must continue to function.

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