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Anophthalmia in a Wild Eastern Gray Squirrel (*Sciurus carolinensis*)

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ABSTRACT: We describe bilateral true anophthalmia in a juvenile female eastern grey squirrel (*Sciurus carolinensis*) with histological confirmation that orbital contents lacked ocular tissues. Additionally, the optic chiasm of the brain was absent and axon density in the optic tract adjacent to the lateral geniculate nucleus was reduced.

In October 2015, a member of the public found a blind juvenile female Eastern gray squirrel foraging on windfall apples in Windsor, Ontario, Canada. The squirrel was submitted to a wildlife rehabilitation facility where it was euthanized and sent to the Canadian Wildlife Health Cooperative (Ontario-Nunavut Region) for autopsy. The squirrel was in good nutritional condition with moderate amounts of visceral and subcutaneous fat stores. The body mass was 261g. Based on body mass (adult squirrels range from 338-750 g) and the presence of a full hair coat, the squirrel was a juvenile between 81 and 94 d of age (Edwards et al. 2003).

The right orbit was completely covered by skin with fused palpebrae (ankyloblepharon; Fig. 1). The left orbit had a 2 mm, truncated palpebral fissure with ventral entropion. Hypoplastic optic nerve roots exited the ventral brain of the affected squirrel but an optic chiasm was absent (Fig. 2A & B).

We examined 4 µm serial sections of H&E-stained orbital contents with light microscopy, finding adipose tissue (occasionally separated by cords of collagen and fibroblasts of varying thickness), skeletal muscle, few clusters of lymphocytes, and ocular adnexal tissues. There was no microscopic evidence of superficial ectoderm (lens), neurocrest (uvea, cornea, sclera, adnexa) or neuroectoderm. Histological examination of the affected brain confirmed the absence of an optic chiasm and the lack of abnormalities in the visual cortex. We stained selected brain sections with combined Luxol fast blue-Holmes. In the region of the lateral geniculate nucleus, where the optic tract originates, the affected brain had markedly fewer axons than a control brain (Fig. 2C & D). We did not detect significant histological abnormalities in tissues of other systems.

The clinical, autopsy, and histological findings lead us to conclude that the ocular abnormalities in this squirrel represent a case of true anophthalmia. Anophthalmia is a rare congenital anomaly characterized by the complete lack of ocular development including the total absence of neuroectodermal tissue in the orbit (Gerth et al. 2013). Other congenital anomalies described in wild squirrels include aortic stenosis (Phillips and Dubielzig 1980), congenital erythropoietic porphyria (Flyger and Levin 1977), and maxillary brachygnathism (Mancinelli and Capello 2016).

Congenital abnormalities of any type, but particularly those involving the eyes, are rarely reported in wildlife. Cases of true anophthalmia in wild mammals that were confirmed with histology include a raccoon (*Procyon lotor*; Render et al. 1983) and white-tailed deer (*Odocoileus virginianus*; Fulton et al. 1977). This may reflect the rarity of this condition and that wild animals simply cannot survive in nature without eyes. In particular, diseased

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small mammals are vulnerable to predation and may be especially challenging for people to detect.

The first remarkable feature of this case is that this juvenile squirrel survived for several months without eyes in an urban area, successfully avoiding potential hazards including predators such as raccoons, domestic cats (*Felis catus*) and dogs (*Canis lupus familiaris*), vehicular traffic, and starvation. Since squirrels are altricial, it was probably safe in the nest until weaning at 56-70 d of age (Edwards et al. 2003). From there, it would have relied upon and developed its capacity to use other senses to navigate its environment for the approximately 11-24 d prior to capture. Based on the presence of fat stores, we presume this squirrel consumed adequate amounts of food, despite the fact that squirrels are highly visual animals (Van Hooser and Nelson 2006). The person that found the squirrel was not aware of anyone providing supplemental food. This finding lends credence to the adage that even a blind squirrel can find a nut.

It is also remarkable that there were no other congenital abnormalities affecting this individual, except in the visual system of the brain. In people and domestic animals, other lesions frequently accompany congenital ocular anomalies. For example, a raccoon affected by anophthalmia also had severe meningoencephalocele, syringomyelia, and hydrocephalus (Render et al. 1983). Similarly, microphthalmia in Japanese brown cattle (*Bos taurus*) was associated with caudal vertebral anomalies (Moritomo et al. 1995). But there are exceptions. For example, in white tailed deer fawns, ocular abnormalities including anophthalmia were not accompanied by other lesions (Fulton et al. 1977). In humans, anophthalmia can be associated with facial, limb, alimentary tract, and central nervous system anomalies (Clementi et al. 1992).

The cause of anophthalmia in this squirrel is unknown and may include genetic, toxicological, infectious, or other teratogenic factors. The neurogenic structures of the vertebrate eye arise from a single pool of progenitor cells in the optic vesicle. Early in embryonic development, the surface ectoderm signals the optic vesicle to invaginate, forming the bilayered optic cup (Matsushima et al. 2011). The optic vesicles remain attached to the prosencephalon by the optic stalks (Sinowatz 2010). Retinal ganglion cell projections are guided by these stalks centrally towards the developing brain, reaching the optic chiasm by day 15 in fetal rats (Lund and Bunt 1976). The apparent hypoplastic optic nerve roots of this case may have been remnants of the optic vesicle buds or stalks, but the lack of optic chiasm or optic tract indicate that these projections either did not form or degenerated.

True anophthalmia are classified as primary or secondary. In primary anophthalmia, germinal primordial optic cells fail to grow from the forebrain and the absence of one or both optic vesicles is the only abnormality. Conversely, secondary anophthalmia occurs when the eyes fail to develop due to anterior neural tube abnormalities, usually caused by an environmental insult in early gestation (Mann 1957). The entire fore- and mid-brain regions develop abnormally, causing other severe gross abnormalities. Based on this classification, this squirrel is an example of primary anophthalmia, which reduces the likelihood of potential environmental teratogen exposure.

It is impractical to distinguish true anophthalmia from severe microphthalmia antemortem; histological confirmation is rarely pursued, and previous descriptions of lesions in the visual system are apparently rare to nonexistent. Although the exact cause is unknown, this case represents a rare congenital anomaly in wildlife, domestic animals, and humans.

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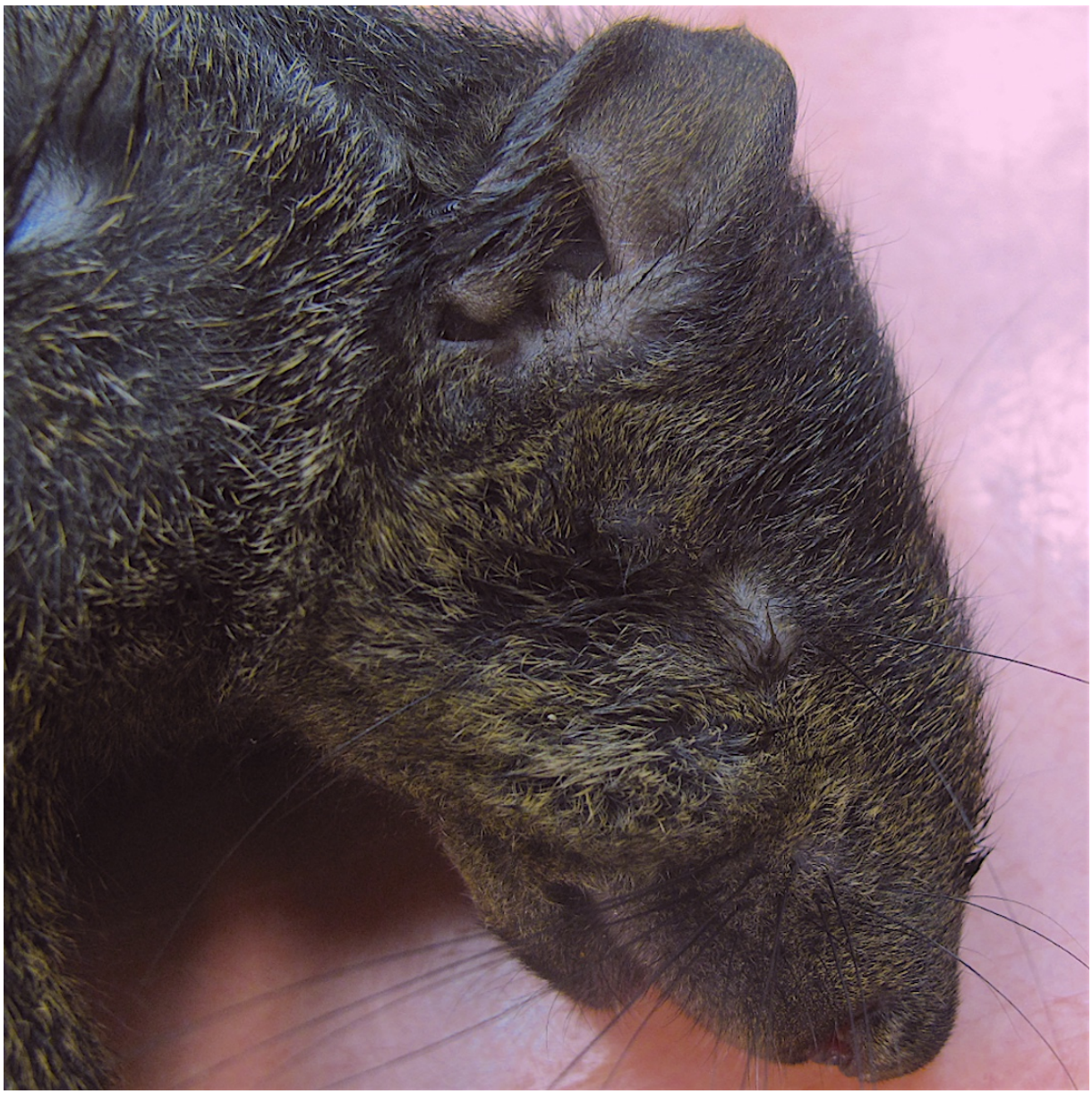

FIGURE 1. Anophthalmia in a wild Eastern gray squirrel (*Sciurus carolinensis)* from Ontario, Canada. The right orbit is covered by a continuous layer of skin with fused palpebrae (ankyloblepharon). Normal vibrissae are adjacent to where the medial canthus is typically located.

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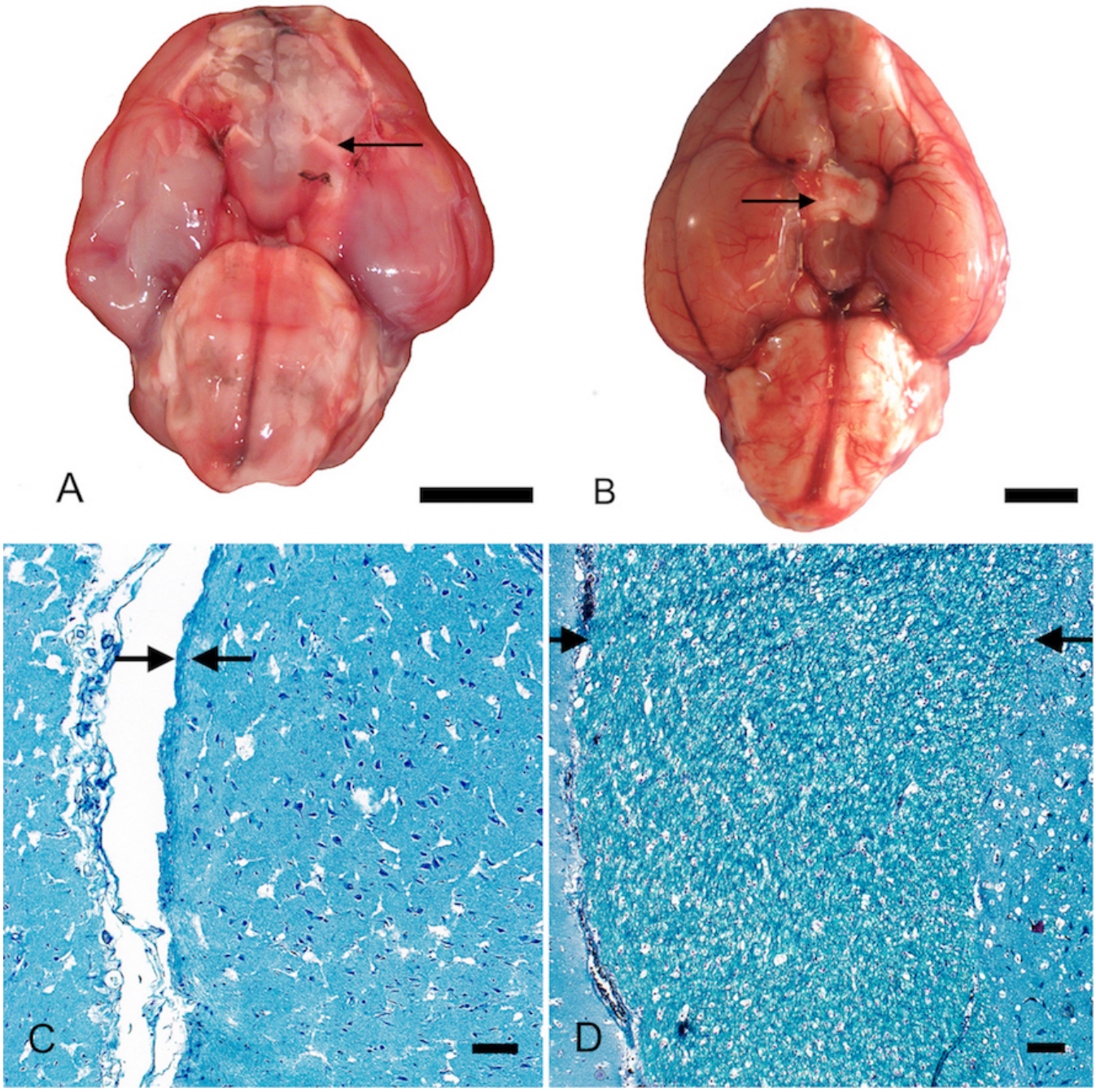

FIGURE 2. Brain tissues from a case of anopthalmia in a wild Eastern gray squirrel (*Sciurus carolinensis*) and a control squirrel from Ontario, Canada. (A) Macroscopic ventral view of the brain of a squirrel affected by anophthalmia. The optic chiasm is absent and optic nerve roots are hypoplastic (arrow). Bar=1 cm. (B) Macroscopic ventral view of the brain of a control squirrel that died of vehicular trauma. The optic chiasm is prominent (arrow). Bar=1 cm. (C) Histology of the optic tract adjacent to the lateral geniculate nucleus in the brain of squirrel affected by anophthalmia. The band of axons (area between arrows) is extremely narrow. Luxol Fast Blue-Homes stain. 10X; Bar=100 µm. (D) Histology of the optic tract adjacent to the lateral geniculate nucleus in the brain of the control squirrel. The band of axons (area between arrows) is markedly wider compared to the case brain. Luxol Fast Blue-Homes stain, 10X; Bar=100 µm.