TECHNICAL NOTE

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Recommended Technique for Brain Removal to Retain Anatomic Integrity of the Pineal Gland in Order to Determine Its Size in Sudden Infant Death Syndrome

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ABSTRACT: A standardized removal and dissection procedure is presented for human infant brain. A previously unreported cistern of the pineal gland must be severed at autopsy in order to preserve the gland's anatomic integrity during brain removal. Utilization of these methods to investigate Sudden Infant Death Syndrome brain tissue should facilitate interdisciplinary studies and comparisons of inter agency findings. We use these dissection procedures to extend our findings on reduced pineal gland size as an anatomic marker assisting the forensic pathologist in making the diagnosis of Sudden Infant Death Syndrome.

KEYWORDS: forensic science, forensic pathology, pineal gland anatomy, Sudden Infant Death Syndrome, brain dissection and removal

The scientific investigation of Sudden Infant Death Syndrome (SIDS) is on the threshold of its first concerted interdisciplinary step. As a diagnosis of exclusion, SIDS is deemed the cause of an infant's death only if no other explanation is identifiable after extensive circumstantial and medical investigation (1). Histologic studies, particularly immunocytochemical investigations using antibodies to transmitter-specific markers, have suggested the need for biochemical investigation (2,3). Therefore, if neurobiochemist, neuroanatomist, and neuropathologist alike are to study similar tissues, then identification of a consistent anatomic marker would aid in correlating data from different institutions.

Pineal gland size has been suggested as such a possible anatomic marker in SIDS. We have shown that the pineal gland of SIDS infants is significantly reduced in size compared with age-matched infants dying of known causes (4). Continued study of pineal gland size as a marker of SIDS required the development of special procedures at necropsy to maintain anatomic integrity of the gland. This evisceration technique is described and applied to assessing the validity of reduced pineal size in SIDS.

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Methods

The recommended brain dissection procedure preserves a previously unreported cistern, which is visible grossly and communicates between the posterior falx and the pineal gland (falcial-parapineal cistern, Fig. 1a). If this cistern is not severed prior to

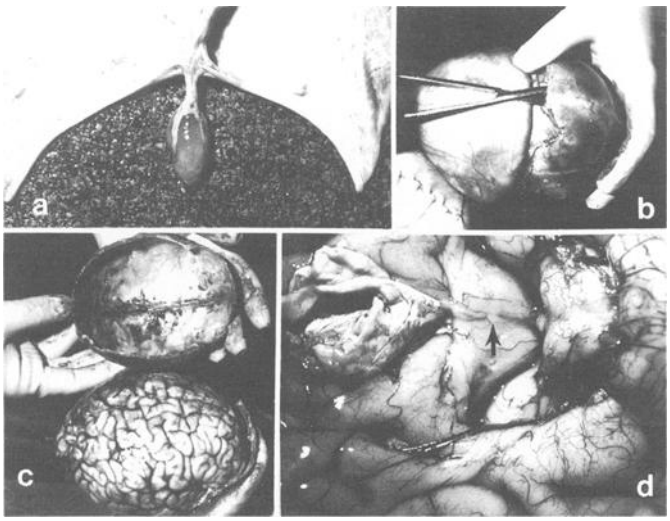


FIG. 1.—*Prosection of the human brain with preserved pineal gland integrity.* a) *The falcial-parapineal cistern is grossly observable in this adult human. The pineal gland has been torn from its central stalk (foreground; posterior commissural and habenular attachments) because of the attachment to the falx via the cistern—which is fluid filled in this individual.* b) *Curved scissors are used to cut the superior aspects of the falx along the superior sagital sinus.* c) *The skull cap is freely removable with minimal disruption of the brain.* d) *The falcial-parapineal cistern has been severed in this human infant brain* (arrow). *The reflected falx and tentorium are to the left and the posterior tip of the pineal gland is to the right nestled between the corpora quadrigeminal plate and the inferior surface of the corpus callosum.*

removing the skull cap, the pineal gland is routinely disrupted from its stalk (CNS attachment) and is, therefore, often unrecognizable after removal of the brain.

Although it is not generally the custom to make a circumferential calvarial cut in a neonatal skull (< one month of age), such a cut can be performed with sharp scissors rather than the striker saw. At this point, it is best that the infant is placed in a upright position so that the brain rests on the base of the skull. The subjacent dura is circumferentially severed with a sharp knife to create separation between the bony edges of the cut skull. The anterior aspect of the skull cap is gently reflected and the falx is incised along the superior sagital sinus utilizing a pair of curved scissors (Fig. 1b). With further gentle lifting, the cut along the superior aspect of the falx is extended to the posterior saw or scissor cut in the skull. The skull cap should be freely removable. The brain *in situ* with the falx essentially intact can be viewed (Fig. 1c). The falx is cut with a sharp knife at its attachment with the midline superior orbital plate (*crista galli*), and gently reflected posteriorly. The posterior cerebral hemispheres are gently displaced laterally to visualize and sever the falcial-parapineal cistern (Fig. 1d).

The prosector next tips the infant's head backwards with the cupped hand as the cerebral support. The olfactory bulbs are detached from the dura, and the optic nerves and internal carotid arteries are transected by sharp knife. The tentorium is severed bilaterally at the posterior aspect of the petrous ridge, as are the cranial nerves at their foramina. The vertebral arteries and the upper cervical cord are then transected, and the brain is gently reflected backwards and withdrawn from the skull.

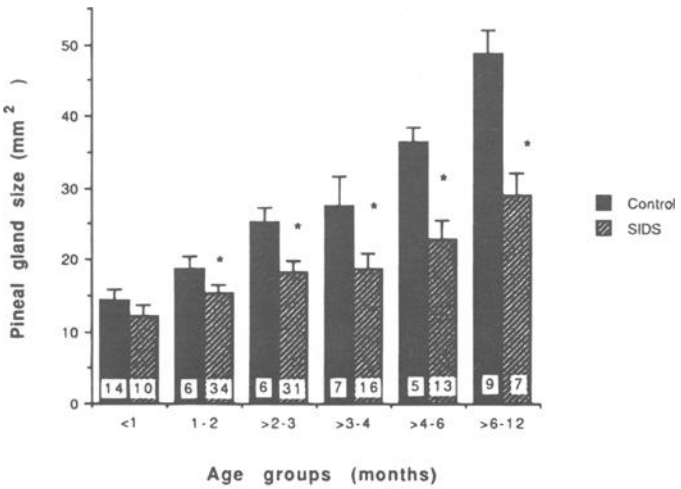
The entire pineal gland and its central stalk are found in the coronal section resulting from sequential incisions made posterior to the mamillary bodies and posterior to the corpus callosum (4). The pineal gland is removed *en bloc* by making two bilateral cuts, each through the cerebral pedulcle and the medial edge of the pulvinar. The pineal gland can then be photographed for morphometric analysis and subsequently investigated by virtue of preservation of gross anatomic integrity.

We used this dissection procedure and previously described morphometric methods (4) to determine the pineal gland size in human infants. The infants were grouped according to those dying of known causes ($N = 47$) and those dying of SIDS ($N = 110$). Within each of these populations, the infants were further partitioned into six groups according to age at death: < 1 month, 1 to 2 months, > 2 to 3 months, > 3 to 4 months, > 4 to 6 months, and > 6 to 12 months.

Results

There was a highly significant ($p < 0.0001$) decrease in the size of the pineal gland in the SIDS population (18.2 ± 0.9 mm^2) compared with the control population (28.0 ± 2.5 mm^2). At the same time, there was no difference in the mean age, brain weight, or body weight between the control and the SIDS populations as a whole.

Further analysis of the data indicated age-related differences between the control and SIDS populations. The size of the pineal gland was not different between the groups under one month of age (Fig. 2). In the one-to-two-month old groups, reduced pineal size in SIDS was significant at the level of a trend, whereas the significance of the reduced size in SIDS increased with increasing age (Fig. 2). Brain weight was significantly greater (20%) in the SIDS infants under one month of age compared with control, but



* = $p < 0.05$ compared with the respective control group.

FIG. 2.—*Pineal gland size in infants dying of known causes and SIDS infants grouped according to age-at-death. The number of infants in each control and SIDS group is noted within the bar. Mean sizes ± SEM are presented.*

was not significantly different in any other age grouping. Body weight was consistently greater in the SIDS groups compared with age-matched controls, but the difference was significant only in the > four-to-six-month-old group.

Discussion

The presented brain removal techniques should assist in performing standardized studies of the pineal gland in human infants by various investigators. Such studies may elucidate an anatomic importance for the previously unreported cistern and could confirm suspicions that the nervi conarii (5) course within the wall of the cistern (G. C. T. Kenny—personal communication). More importantly, these investigations could establish reduced pineal gland size as an anatomic marker to assist the pathologist in reaching the diagnosis of Sudden Infant Death Syndrome. Furthermore, reduced pineal gland size could be involved in the mechanism of a SIDS death. Reports of increased oxidative stress (6) and neurodegeneration in SIDS brain (3,7) may be related to decreased circulating levels of the anti-oxidant melatonin—a neurohormone produced primarily by the pineal gland (8,9).

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