source. In this regard, we have found no evidence of nucleotide sequence homology between VRV cDNA and cellular DNA's of a number of primate species. Similarly, cDNA probes synthesized from primate type D retroviruses have not hybridized with cellular DNA's of reptilian species so far examined. If such homology were demonstrated, it might provide evidence of relatively recent virus transmission from one line to the other. More extensive analysis will be necessary to determine whether information related to either virus group can be detected within the genomes of species representing other vertebrate families. It is also possible that type D viruses became endogenous to vertebrates prior to the divergence of lines leading to reptiles and mammals more than 300 million years ago. If so, the antigenic determinants shared by the major structural proteins of these viruses would have to be extremely well conserved (*12*). In any case, our studies establish type D retroviruses as a group whose distribution among vertebrates may even be wider than that of type C RNA viruses.

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Localization of the Neurally Mediated Arrhythmogenic Properties of Digitalis

Abstract. *Available evidence suggests that the propensity of digitalis glycosides to produce cardiac arrhythmias is due in part to their neuroexcitatory effects. We have performed experiments in cats which support the existence of a neurogenic component in the etiology of digitalis-induced ventricular arrhythmias. Our data further indicate that the locus of this neural effect lies within an area of the medulla 2 millimeters above to 2 millimeters below the obex. These findings, when considered with the effects of polar cardiac glycosides that do not cross the blood-brain barrier, suggest that the area postrema may be the site of neural activation.*

The cardiac glycosides possess the ability to enhance cardiac contractility and to control certain cardiac rhythm disturbances. These drugs also increase peripheral vascular resistance and, at high doses, may produce emesis, alterations in color vision, and hyperventilation (*1*). The latter phenomena are all thought to be due to the neuroexcitatory properties of digitalis (*2*). The most significant manifestation of digitalis toxicity is alteration of cardiac rhythm. Although digitalis clearly exerts arrhythmogenic effects by direct action on the heart, neural influences have been suggested to play a significant role in the facilitation of cardiac arrhythmias caused by digitalis (*3*).

Transection of the spinal cord at the atlanto-occipital junction (C-1) protects against digitalis toxicity, as judged by higher cumulative drug dose and myocardial content of glycoside at onset of cardiac toxicity. This has been cited as evidence for the nervous system's role in digitalis-induced cardiotoxicity in the cat (*4*). However, the resulting reduction in heart rate and arterial blood pressure by C-1 section causes uncertainty about the mechanism of this protection, since a decrease in myocardial blood flow could protect by decreasing myocardial drug delivery.

Evidence exists to indicate that inhibition of the monovalent cation transport enzyme sodium- and potassium-activated adenosinetriphosphatase is responsible for the direct toxic effects of digitalis on the heart (*5*). We have tried to define further the significance of the higher cumulative ouabain dose and myocardial content of ouabain found at onset of overt toxicity after spinal cord section by measuring active transport of the K^+ analog Rb^+ in myocardial samples from neurally intact and C-1 spinal cord–sectioned animals.

In pentobarbital-anesthetized (30 mg per kilogram of body weight) cats, C-1 spinal cord section increased the dose of the cardiac glycoside ouabain (infused at 1 μg per kilogram of body weight per minute) needed to produce ventricular tachycardia (VT). In a series of 12 experiments, C-1 section increased the dose of ouabain from 76 ± 4 (mean $\pm$ standard error) to 114 ± 8 μg per kilogram of body weight, and decreased the active transport of Rb^+ at onset of VT from 0.41 ± 0.04 to 0.24 ± 0.01 nmole per milligram of wet tissue weight in 30 minutes ($P < .01$). These data indicate that removal of neural influences on the heart by cord section at the level of the atlanto-occipital junction permits 50 percent more drug to be administered before onset of cardiac arrhythmias. Although spinal section caused a mean drop of arterial blood pressure of 57 percent, reduced myocardial blood flow and ouabain delivery do not account for this protective effect, since inhibition of active Rb^+ uptake at onset of VT was increased by 41 percent. A more marked inhibition

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of myocardial monovalent cation active transport at onset of digitalis toxicity with the sympathetic nervous system's link to the heart severed (*6*) confirms the protective effect of withdrawing sympathetic neural input.

We then used Rb^+ transport to measure the effect of digitalis on myocardial monovalent cation transport at different neural states. We employed the aminosugar cardiac glycoside, 3-β-O (4-amino-4, 6-dideoxy-β-D-galactopyranosyl) digitoxigenin (ASI-222), a polar semisynthetic cardiac glycoside derivative that does not cross the blood-brain barrier, as judged by the absence of detectable levels in the cerebrospinal fluid (CSF) by a radioimmunoassay method sensitive to $2 \times 10^{-10} M$ concentrations (*7*). We reasoned that a cardiac glycoside unable to gain access to the CSF might not precipitate digitalis-like neural activation, and thus might be less likely to cause toxic effects. However, as in the case of ouabain, C-1 section increased the dose of ASI-222 (infused intravenously at 1 μg per kilogram of body weight per minute) needed to produce VT in cats from 87 ± 4 to 116 ± 8 μg per kilogram of body weight, and decreased the active transport of Rb^+ at VT onset from 0.42 ± 0.03 to 0.23 ± 0.02 nmole per milligram of wet tissue weight in 30 minutes ($P < .01$). Thus, C-1 section also affords protection against a polar glycoside that lacks access to portions of the central nervous system (CNS) with an effective blood-brain barrier. These findings suggested that the neuroexcitatory locus of digitalis action that is responsible for potentiating cardiac arrhythmias lay in an area or areas of the CNS rostral to the C-1 level, but not protected by the blood-brain barrier.

Table 1. The effect of neuraxis transection on ouabain dose and inhibition of myocardial monovalent cation transport at onset of ventricular tachycardia (VT). Ouabain was infused at 1 μg per kilogram of body weight per minute to each group of six cats. Values given are expressed as mean ± standard error. Onset of VT was defined as the initial appearance of 1 minute of a sustained tachyarrhythmia with atrioventricular dissociation and broadened QRS complexes on electrocardiograms.

Site of neural section	Dose of ouabain to VT (μg/kg)	Rb^+ active transport (nmole/mg wet weight per 30 min)*
None	78 ± 3	0.34 ± 0.04
Mid-collicular	81 ± 2	0.32 ± 0.03
Above obex	76 ± 6	0.34 ± 0.02
Below obex	128 ± 9†	0.22 ± 0.02†
C-1	117 ± 9†	0.19 ± 0.01†

*Left ventricular myocardial slices (ten samples per group) approximately 1 by 2 by 5 mm in size were obtained immediately at onset of VT and were incubated at 30°C for 30 minutes in physiologic buffer containing 4.0 mM K^+ and 0.1 mM Rb^+, with radioactive $^{86}Rb^+$ present as tracer (10^5 cpm/ml). The active transport was determined as the difference between total Rb^+ uptake and that observed in the presence of $10^{-3} M$ ouabain (*15*). †Significantly different from the neurally intact group, the mid-collicular section group, and the above obex section group ($P < .01$).

We undertook experiments to localize this site using pentobarbital-anesthetized (30 mg per kilogram of body weight), mechanically ventilated, vagotomized cats; all other nerves to the heart were intact (*8*). Ouabain infused intravenously at a rate of 1 μg per kilogram of body weight per minute produced VT at a mean cumulative dose of 78 μg per kilogram of body weight (Table 1). Complete transection of the neuraxis at the mid-collicular level or 2 mm above the obex (see Fig. 1 for anatomic orientation) changed neither the dose of ouabain required to produce VT nor the myocardial active transport of Rb^+ at this endpoint. As summarized in Table 1, however, brain stem transection 2 mm below the obex increased significantly the dose of ouabain needed to produce VT. The increase observed was indistinguishable from that caused by C-1 spinal cord transection. Inhibition of active Rb^+ transport at onset of VT was significantly greater than in neurally intact preparations, and again produced findings indistinguishable from spinal cord transection at C-1.

These results indicate that the site of digitalis-induced neural activation responsible for facilitation of cardiac rhythm disturbances in the cat (*9*) lies in an area in the lower brain stem within 2 mm of the obex. The area postrema, gelatinous area, underlying reticular formation, and nucleus intercalatus (*10*) (Fig. 2), as well as other structures near the floor of the fourth ventricle, could be responsible for the facilitation of cardiac arrhythmias, or could mediate the linkage of afferent neural activation to efferent sympathetic discharge (*11*). Areas close to the ventricular system are especially likely, in view of experiments showing that instillation of small amounts of cardiac glycosides into the third and fourth ventricles of the brain produced cardiac arrhythmias (*12*). We believe that a likely locus of digitalis-induced arrhythmogenic activity is the area postrema, known to subserve the function of a chemoreceptor trigger zone (*13*). The area postrema comprises a por-

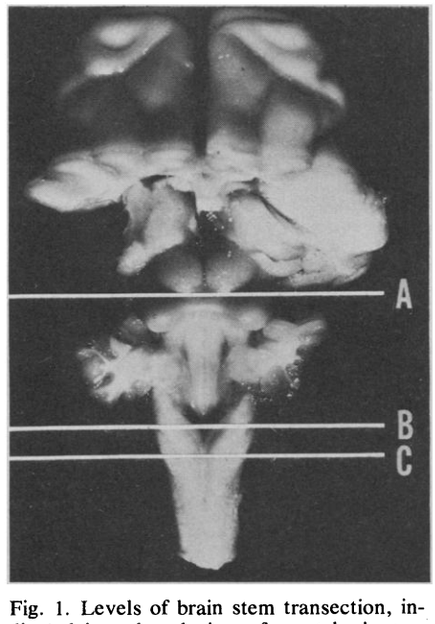

Fig. 1. Levels of brain stem transection, indicated in a dorsal view of a cat brain preserved with 10 percent formalin injected intra-arterially. The cerebellum has been removed to reveal the colliculi and midbrain. (A) Mid-collicular level of section; (B) section level above obex; (C) section level below obex. Transection was performed by surgical exposure of the dorsal aspect of the brain stem followed by complete section through the brainstem at the levels described. At the end of each experiment, anatomical verification of the completeness of the transection was made.

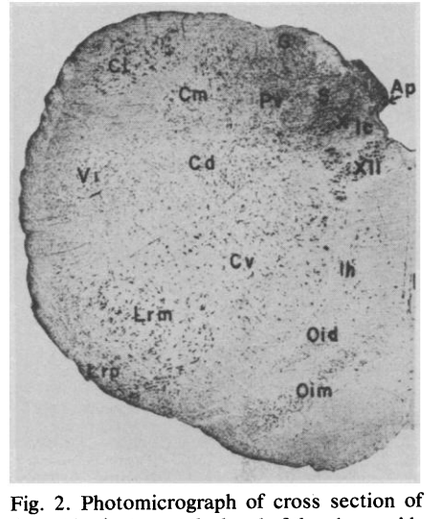

Fig. 2. Photomicrograph of cross section of the cat brainstem at the level of the obex, midway between (B) and (C) in Fig. 1. Abbreviations: *Ap*, area postrema; *Cd*, nucleus medullae oblongatae centralis, subnucleus dorsalis; *Cl*, nucleus cuneatus lateralis; *Cm*, nucleus cuneatus medialis; *Cv*, nucleus medullae oblongatae centralis, subnucleus ventralis; *G*, nucleus gracilis; *Ic*, nucleus intercalatus; *Ih*, nucleus interfascailaris hypoglossi; *Lrm*, nucleus lateralis reticularis, subnucleus magnocellularis; *Lrp*, nucleus lateralis reticularis, subnucleus parvocellularis; *Oid*, nucleus olivaris inferior accessorius dorsalis; *Oim*, nucleus olivaris inferior accessorius medialis; *Pv*, nucleus parvocellularis compactus; *S*, nucleus tractus solitarii; *Vi*, nucleus tractus spinalis trigemini interpolaris; *X*, nucleus nervi vagi dorsalis motorius; *XII*, nucleus nerve hypoglossi. [Courtesy of and modified from E. Taber Pierce (*14*)]

tion of the sides and floor of the fourth ventricle, forming the dorsal landmark known as the obex (*14*). The highly vascular area postrema lacks a blood-brain barrier (*14*), and has been shown by Borison and Wang (*13*) to mediate the emetic effects of digitalis. Preliminary studies demonstrate that the area postrema may affect circulatory hemodynamics, presumably through the autonomic nervous system (*14*). Activation of this area by digitalis glycosides would be consistent with the effects observed in the lesioning experiments and also with the apparent neural facilitation of arrhythmias by ASI-222, a polar cardiac glycoside that does not cross the blood-brain barrier in detectable amounts.

The experiments described here confirm the appreciable influence exerted by neural factors in the facilitation of digitalis-induced cardiac arrhythmias. Further, these studies define an area in the medulla within 2 mm of the obex as the locus for the neurally mediated arrhythmogenic properties of digitalis.

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Delay of Constant Light–Induced Persistent Vaginal Estrus by 24-Hour Time Cues in Rats

Abstract. *The normal ovarian cycle of female rats is typically replaced by persistent estrus when these animals are housed under constant light. Evidence presented here shows that the maintenance of periodicity in the environment can at least delay (if not prevent) the photic induction of persistent vaginal estrus. Female rats in constant light were exposed to vaginal smearing at random times or at the same time every day. In another experiment, female rats were exposed to either constant bright light, constant dim light, or a 24-hour photic cycle of bright and dim light. The onset of persistent vaginal estrus was delayed in rats exposed to 24-hour time cues even though the light intensities were the same as or greater than those for the aperiodic control groups. The results suggest that the absence of 24-hour time cues in constant light contributes to the induction of persistent estrus.*

When female albino rats are placed in constant light, their 4-day estrous cycle of sexual receptivity, ovulation, and vaginal cornification is replaced over several weeks by continuous sexual receptivity, chronic vaginal cornification, and cessation of ovulation (*1-3*). This persistent estrus could be attributed either to the increase in the amount of light as such, or to the absence of strong 24-hour time cues, or to both. An explanation solely in terms of amount of light has been favored for two reasons. First, rats do not develop persistent estrus in constant darkness despite the fact that strong 24-hour time cues are absent (*1*, *4*). Second, an increase in the intensity of constant light speeds the onset of persistent estrus (*5*).

Despite the importance of light intensity, the absence of 24-hour time cues in constant light could interact with light to induce persistent estrus. Chen and Besch (*6*) have provided some support for this hypothesis. Rats in a cycle of 2 hours of light alternating with 2 hours of darkness (LD 2:2), a schedule that does not synchronize the rat's circadian activity cycle (*7*), showed lengthened estrous cycles with increased vaginal cornification compared to rats in a LD 12:12 cycle even though the total amount of light was the same in both conditions.

If the absence of 24-hour time cues contributes to the induction of persistent estrus in constant light, then adding such time cues should delay the onset of persistent vaginal estrus. In the present study 24-hour time cues were added in two ways: by taking vaginal smears at the same time each day in experiment 1, and by superimposing 12 hours of bright light on constant dim light every 24 hours in experiment 2. Onset of persistent estrus was defined as the first day of eight or more consecutive smears showing cornified or nucleated vaginal epithelial cells (*8*).

In experiment 1, 18 female rats were exposed to constant light (35 lux) for 53 days. Vaginal smears were taken from half the animals at the same time (1100) 6 days a week and from the other half at random times 6 days a week (*9*). Because periodic noise can be an effective time cue for synchronizing the activity cycle of some rats (*10*), the two groups were housed in separate rooms that were entered only to take vaginal smears.

As shown in Fig. 1, rats subjected to 24-hour periodic vaginal smearing developed persistent estrus more slowly (median onset, 44 days) than rats subjected to random vaginal smearing (28 days) ($P < .05$, Mann-Whitney U test, one-tailed). Although both groups were exposed to the same amount of light and vaginal smearing, the group with the 24-hour nonphotic time cue maintained estrous cyclicity longer.

In experiment 2, eight rats were ex-

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