

## Developments in Basic Neurolaryngology

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## Neurolaryngology

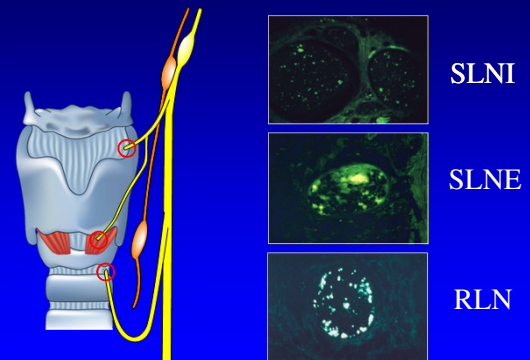
- The regulation of the activity of the larynx in respiration, deglutition and phonation naturally demands delicate innervation systems
  - Many basic researchers perform studies in otology in our fields, basic researchers rarely undertake a study of the larynx
- Medical doctors like myself are forced to do the basic research in the laryngology by ourselves

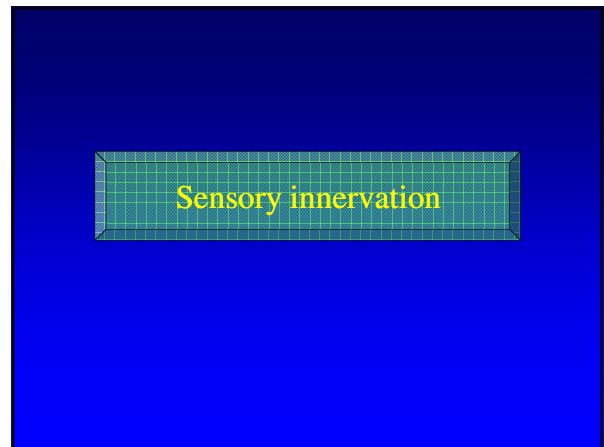
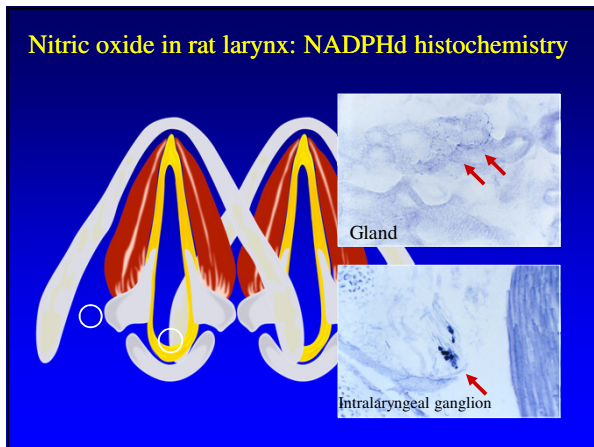
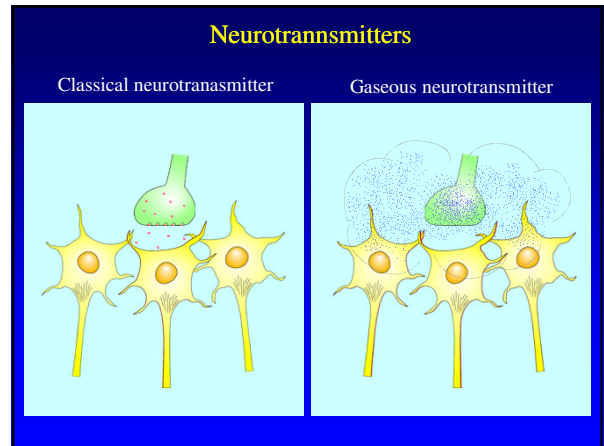
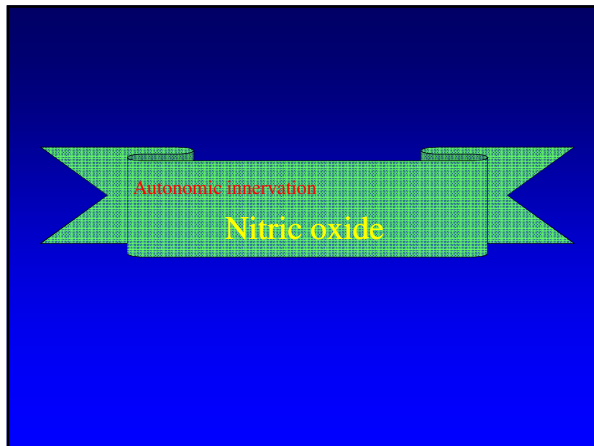
## Autonomic innervation

## Autonomic innervation

- The autonomic innervation participates in the regulation of glandular secretion and blood circulation of the larynx
- Laryngeal secretion is essential for the maintenance of laryngeal function mainly lubricating the vocal folds for phonation and contributing the local immunological mucosal defense system

## Canine laryngeal nerves : Falck-Hillarp





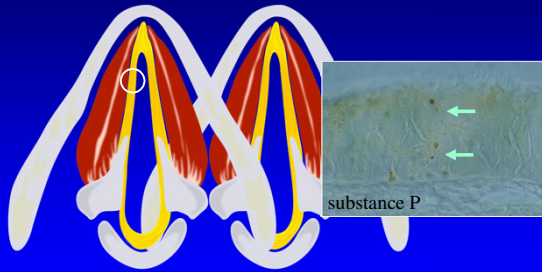
**Sensory innervation**

- Sensory innervation is essential in the laryngeal defense system including laryngeal reflex

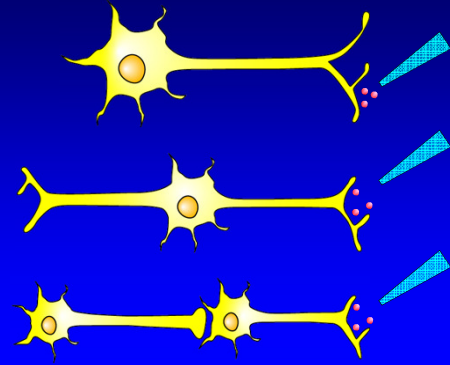
**Neuropeptide**

- Several kinds of neuropeptides are putative sensory neurotransmitters and/or neuromodulators in laryngeal innervation

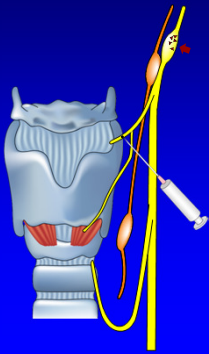
### Neuropeptide in the canine laryngeal mucosa



### Neurotracing technique



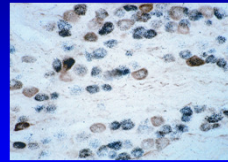
### Neuropeptide in the laryngeal sensory neurons



- Neurotracer injection into the internal branch of canine superior laryngeal nerve
- Detection of neuropeptide in the labeled neurons in the NG by immunohistochemistry

### Neuropeptide in the laryngeal sensory neurons

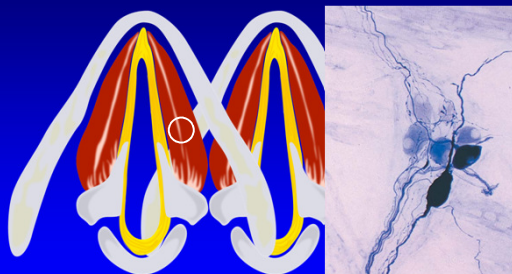
#### Nodose ganglion



CGRP : 81.5%  
 substance P : 24.5%  
 enkephalin : 7.0%

Neurotracer + CGRP immunohistochemistry

### Sensory neurons in the intrinsic muscles



NO positive cells

Sensory innervation

**Nociceptors**

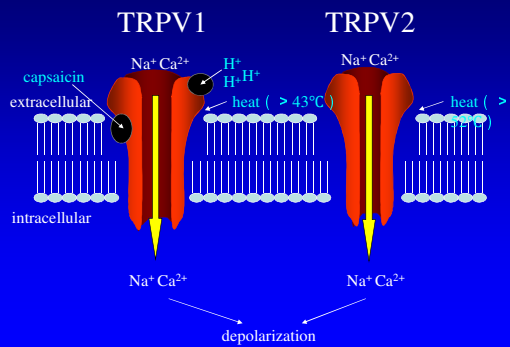
## Sensory receptors

mechanoreceptor  
chemoreceptor  
photoreceptor  
thermoreceptor  
osmoreceptor  
nociceptor

## Nociceptors

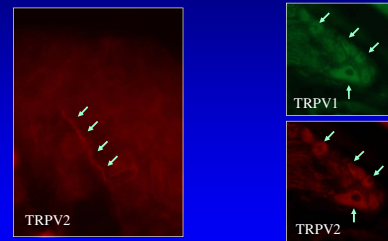
	subtype	activator
Vanilloid receptor	TRPV1	Capsaicin
	TRPV2	Heat
	TRPV3	H <sup>+</sup>
	TRPV4	H <sup>+</sup>
	TRPV5/6	
ATP receptor	P2X <sub>2</sub> P2X <sub>3</sub>	ATP
Acid-sensing ion channels	ASIC1 ASICβ ASIC3 ASIC2b	H <sup>+</sup>

## Activating process of vanilloid receptor



## Vanilloid receptors in the rat laryngeal mucosal epithelium and intralaryngeal ganglion

Coexistence in most cells

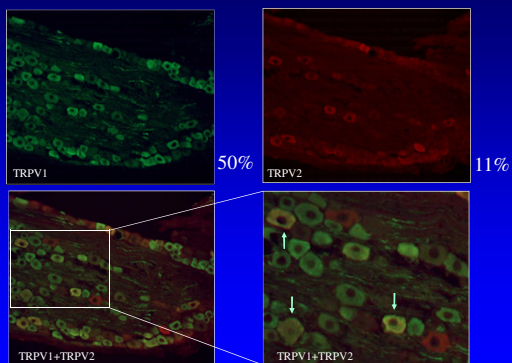


Epithelium

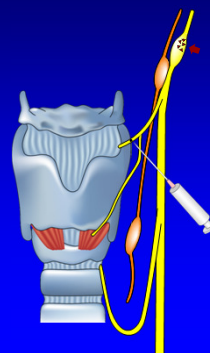
Intralaryngeal ganglion

Some intraganglionic neurons have sensory function

## Vanilloid receptors in the nodose ganglion



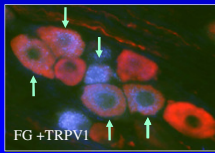
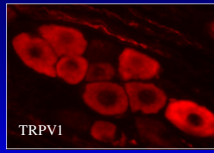
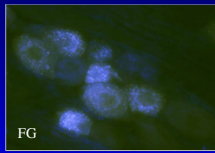
## Laryngeal sensory neurons participating in the nociception



Injection of FluoroGold into the SLNI

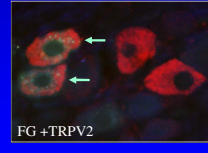
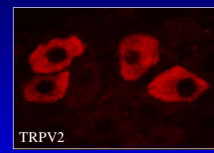
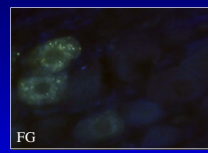
The number of vanilloid receptor positive neurons among the laryngeal sensory neurons in the NG was counted

### TRPV1 expression in the laryngeal sensory neurons



- 49% of the laryngeal sensory neurons in the NG are TRPV1 positive

### TRPV2 expression in the laryngeal sensory neurons



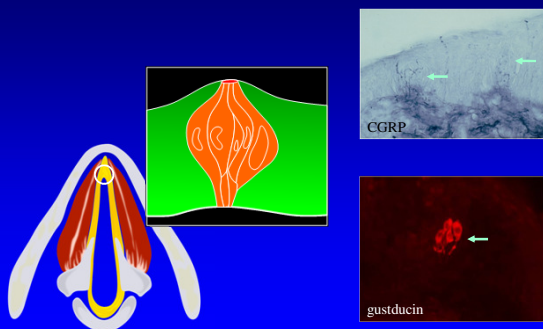
- 12% of the laryngeal sensory neurons in the NG are TRPV2 positive

### Vanilloid receptors in the laryngeal innervation

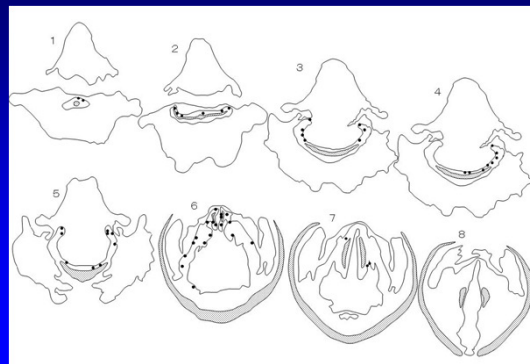
- The ratio of TRPV1 or TRPV2 positive neurons among the laryngeal sensory neurons is almost the same as the ratio among the general population of NG neurons
- Both TRPV1 and TRPV2 participate in neurotransmission of nociception in the larynx, with TRPV1 having a major role



### Taste receptors in the larynx



### Taste receptors in the larynx



## Sensory receptors

- Vanilloid receptors were found in the laryngeal mucosa and nodose ganglion, and may participate in neurotransmission of laryngeal nociception
- Gustducin positive cells were found in taste-bud like structures of the larynx, and may function as taste receptors

## Motor innervation

## Motor innervation

- The regulation of the activity of the intrinsic laryngeal muscles demands delicate innervation system
- In these systems, the location of motoneurons innervating the intrinsic laryngeal muscle is important
- The regulation system of the laryngeal motoneurons was not clearly detected

## Motor innervation

- The laryngeal motoneurons which innervate intrinsic laryngeal muscles are located in nucleus ambiguus (nAmb)
- The nAmb is situated in the ventral part of the medulla oblongata and exhibits long rostrocaudal extension
- Laryngeal motoneurons are scattered in nAmb  
→It is difficult to selectively inject a neuronal tracer to laryngeal motoneurons

Motor innervation

Central projections to laryngeal motoneurons

## Transneuronal labeling

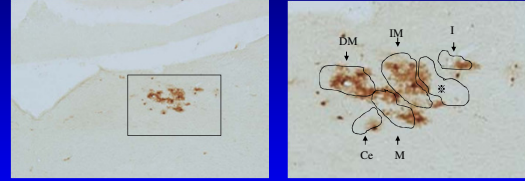
- Herpes simplex virus (HSV) can invade the central nervous system after inoculation into peripheral target organs
- HSV type-2 (HSV-2) is known to be one of the effective transneuronal tracer with its neurotropic nature
- We used HSV-2 as transneuronal tracer in this experiment

## Transneuronal labeling study

Injection of HSV-2 into mice intrinsic laryngeal muscles #1  
 ↓  
 Whole brain was removed  
 ↓  
 Serial frozen sections of 20 mm thickness  
 ↓  
 Immunohistochemistry  
 primary antibody: rabbit anti HSV-2 antibody  
 secondary antibody: biotinylated anti rabbit IgG

#1 cricothyroid (CT) muscle  
 thyroarytenoid/lateral cricoarytenoid (TA/LCA) muscle

## Nucleus of solitary tract : CT

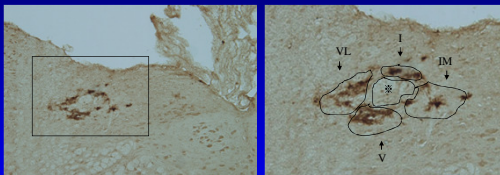


Subnucleus of nucleus of solitary tract

Ce: central, DM: dorsomedial, I: interstitial, IM: intermediate, M: medial

※: solitary tract

## Nucleus of solitary tract : TA/LCA

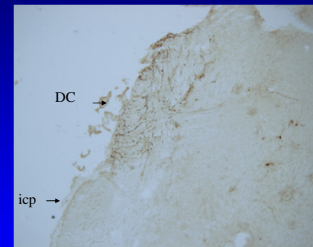


Subnucleus of nucleus of solitary tract

I: interstitial, IM: intermediate, V: ventral, VL: ventrolateral

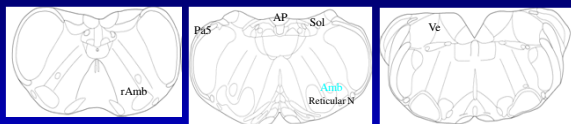
※: solitary tract

## Cochlear nucleus (C) :TA/LCA

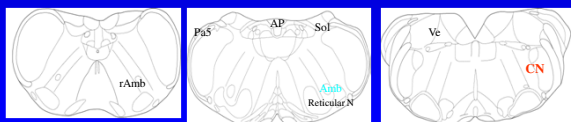


DC: dorsal C, icp: inferior cerebellar peduncle

## CT



## TA/LCA



## Central projection to laryngeal motoneurons

- No direct connections were recognized between bilateral nAmb
- No direct projection was recognized from periaqueductal gray matter (PAG) or cortex

### Central projection to laryngeal motoneurons

retroambiguus n.	: phonation
NST	: sensory input
reticular n.	: respiration and swallowing
paratrigeminal n.	: circulation and respiration
vestibular n.	: vestibular respiratory reflex
area postrema	: emesis
cochlear n.	: auditory input to phonation

- Intrinsic laryngeal muscles have multiple ways of input
- The present results indicate the complexity of laryngeal function

### Current topics

### Circadian rhythm in the larynx

### Disorders related to circadian rhythm

- Hypertension
- Myocardial infarction
- Brain infarction
- Gastric ulcer
- Psychiatric disorders
- Rheumatoid arthritis
- Airway disorders

### Larynx

The tone of voice is delicately different between daytime and night

Midnight worsening of cough is a frequent complaint of patients with laryngitis and bronchitis, and acute subglottitis mainly occurs in the night

### History of circadian rhythm research

#### Circadian: Circa=about, dies=a day

1792 Circadian rhythm was detected in leaves of plants

1945 Body temperature showed 25 hour rhythm in an isolated air-raid shelter

1971 Rhythmic disorder was discovered in fruit fly  
Period gene was identified later

### History of circadian rhythm research

1970s Suprachiasmatic nucleus (SCN) was identified as the master clock  
(using a SCN destruction model)

1984 Fruit fly clock gene "Period" was cloned

1997 Mammalian clock genes "Clock", "Per" were cloned

## Peripheral clock in the airway

If circadian rhythm exists in the airway, mucous secretion is the most probable target

Is there a rhythmic change of mucous secretion in the airway?

## Rhythm in laryngeal mucous secretion

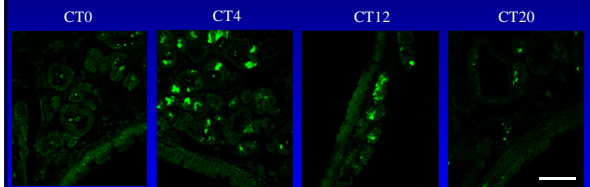
Muc5b, the gene of airway mucin, was measured by immunohistochemistry to assess circadian change of mucous content in the murine larynx

## Circadian time

Mice were housed in an environment with a 12-hr light and 12-hr dark cycle for 2 weeks, then moved to constant darkness

CT0-12 Subjective Day  
 CT12-24 Subjective Night  
 ex) CT0: 6:00 CT12: 18:00

## Circadian expression of Muc5b in laryngeal glands



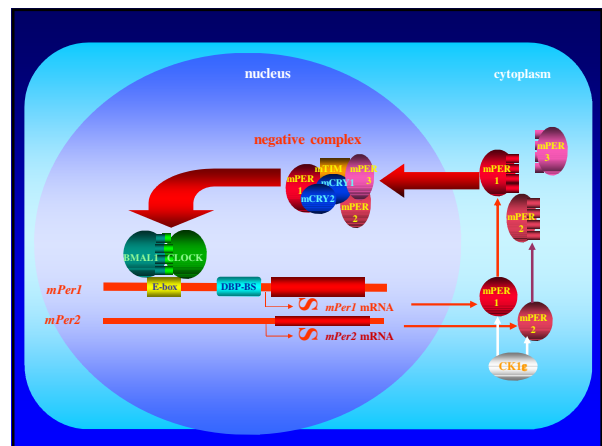
Scale bar : 100µm

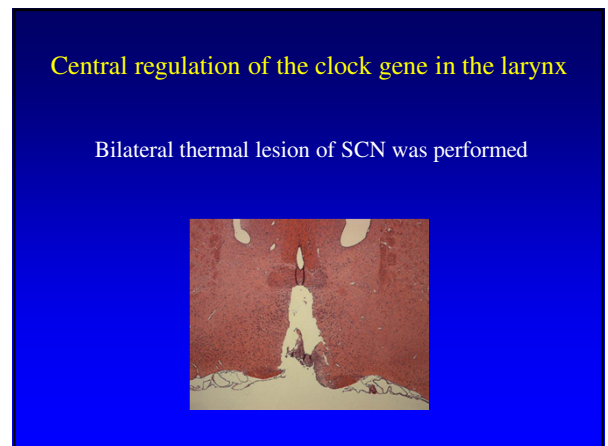
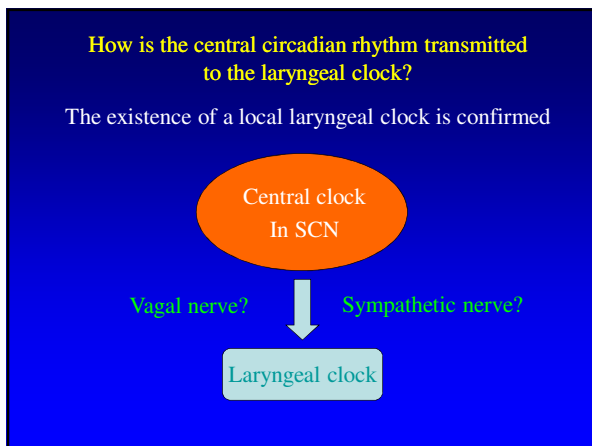
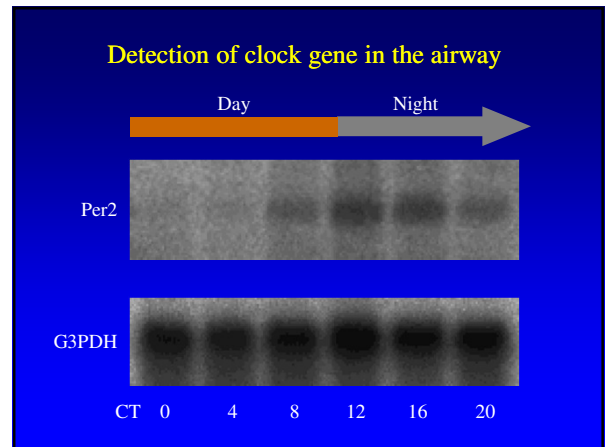
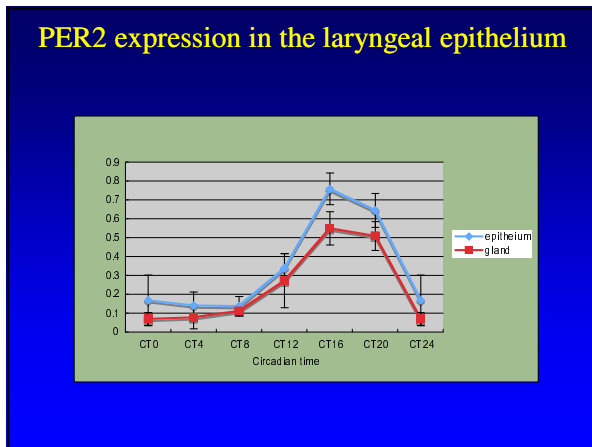
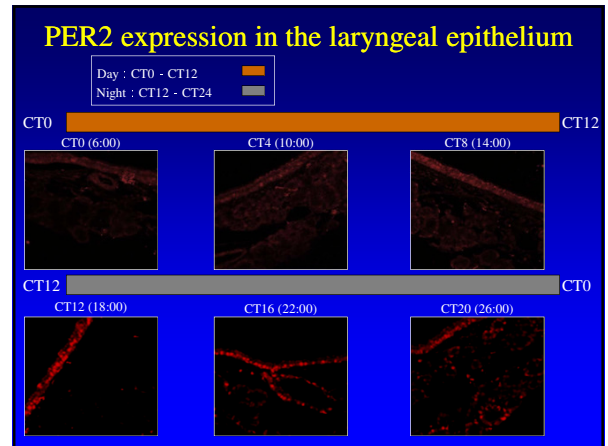
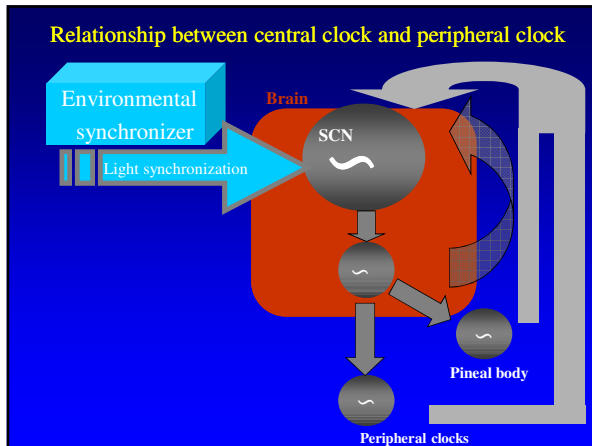
Circadian rhythm was confirmed in the laryngeal secretion

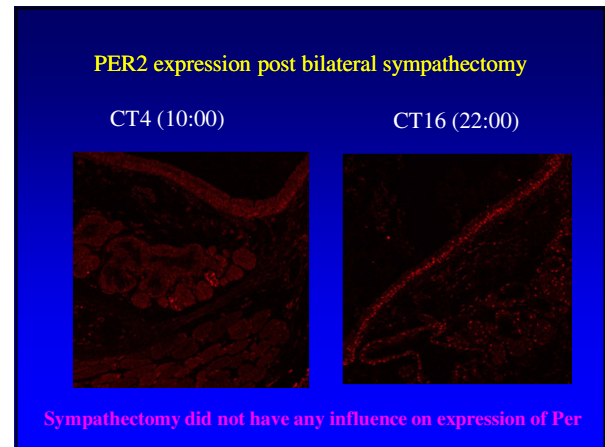
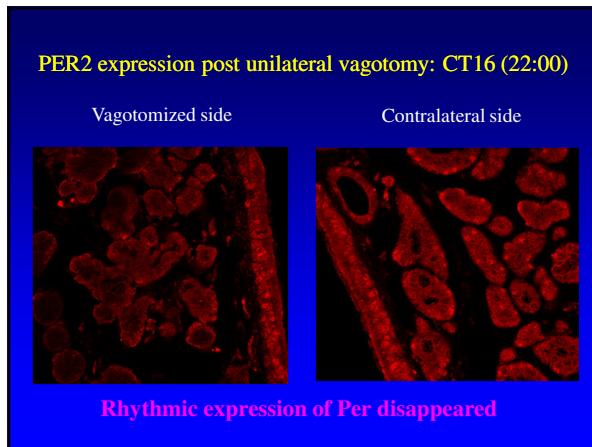
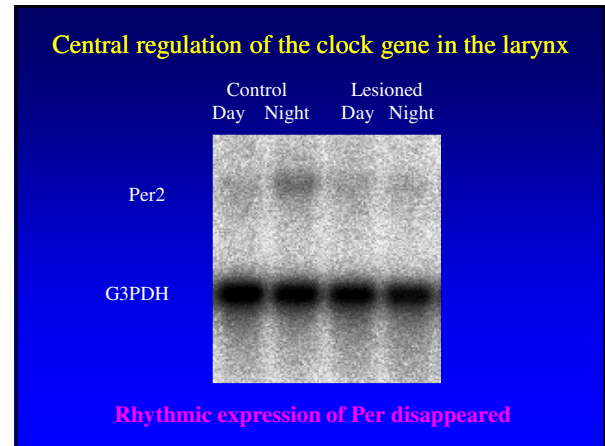
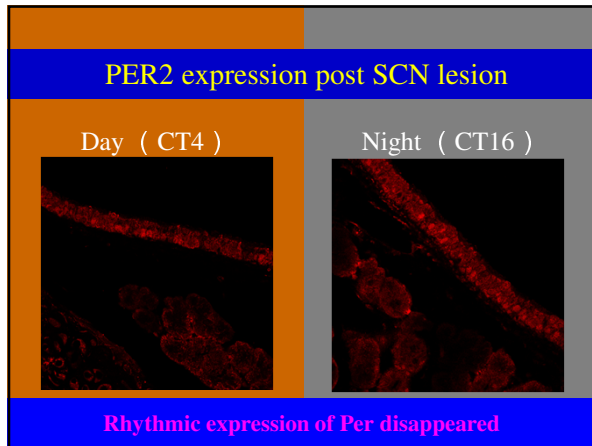
## Mammalian clock gene family

Per (Per1, Per2, Per3) main oscillator genes  
 Cry (Cry1, Cry2) negative elements  
 Bmal1 positive element  
 Clock positive element

Genetic lesion of these genes causes rhythmic disorders







**Transmission of the regulation**

These results suggest that the vagus nerve transmits circadian time originated in the SCN to the larynx

**Future perspectives**

The peripheral clock has been shown to control laryngeal secretion

The peripheral clock is a potential target for future therapeutic schemes of both benign and malignant disorders of the larynx

