

EPIDEMIOLOGY and CLINICAL SIGNS ASSOCIATED with ACUTE and LONG TERM EFFECTS of DOMOIC ACID TOXICITY in MARINE MAMMALS ALONG the WEST COAST of NORTH AMERICA

Martin Haulena (and a lot of borrowed stuff from a lot of people)

Tick CSL 3719

- Adult female (65.5)
California sea lion in good
body condition stranded
May 21, 1998
- Seizures, obtunded, other
similar sea lions stranding
at same time
- WBC / serum chemistry
 - Eosinophils = 9%
- Differential diagnoses
 - Organophosphate toxicity,
infectious encephalitis
- Given lactated Ringer's
and penicillin



Neurological signs

Ataxia, head weaving, muscle tremors

Opisthotonus

Seizures (tonic/clonic)

Status epilepticus



Abnormal behavior

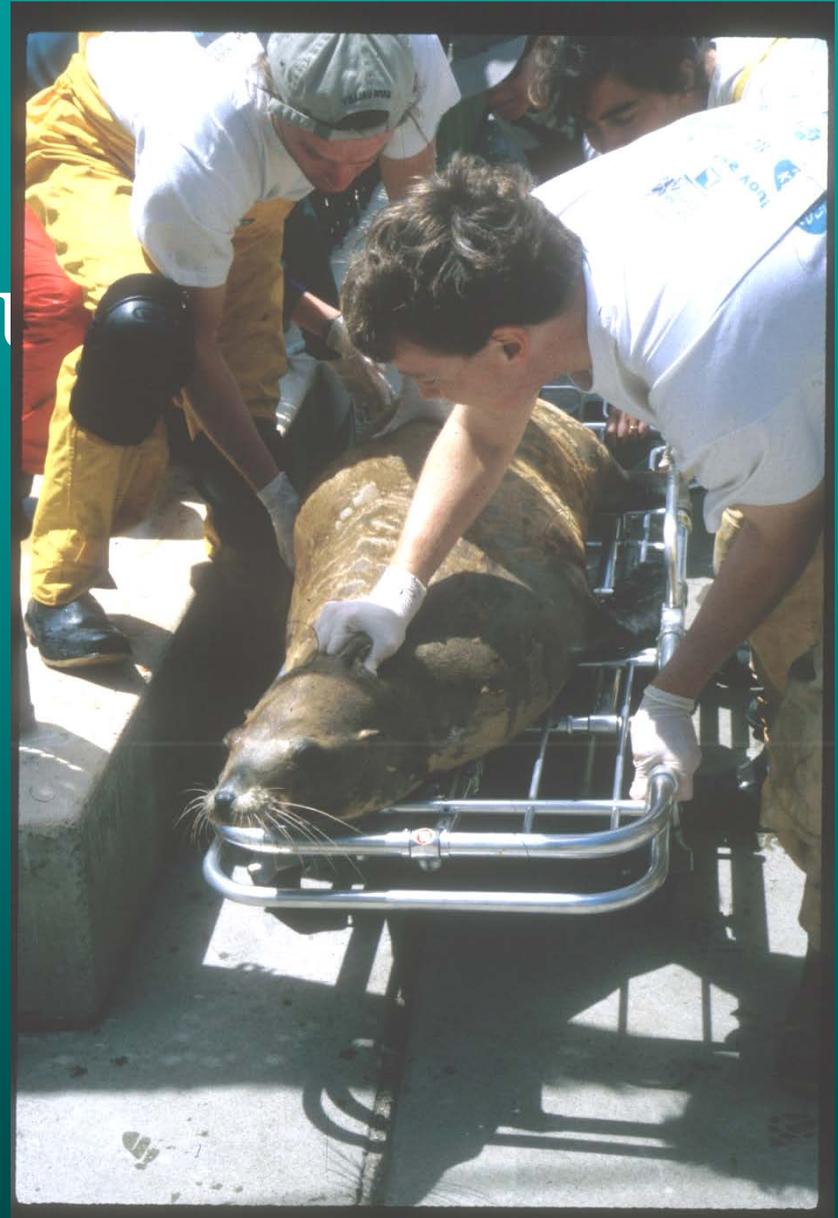
Dull, lethargic, weak,

-approachable

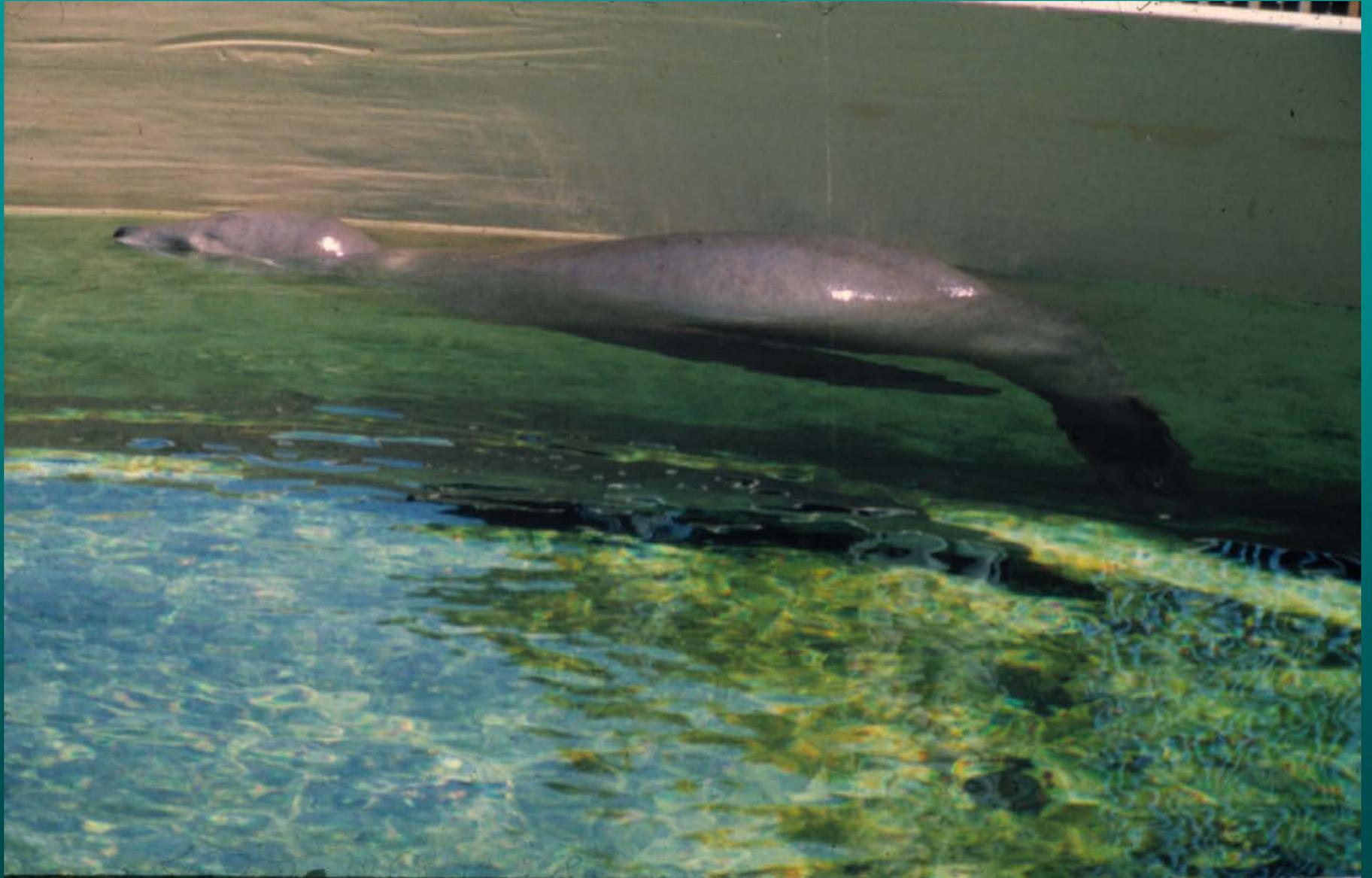
**-able to take blood samples
without restraint**

Rubbing

Floating in pools



Floating in pools



Blood draw without restraint



Domoic acid toxicity outbreak

- Given thiamine injection 450 mg IM
- Gave birth to live pup but did not nurse (pup placed at a display facility)
- Eventually recovered
- released



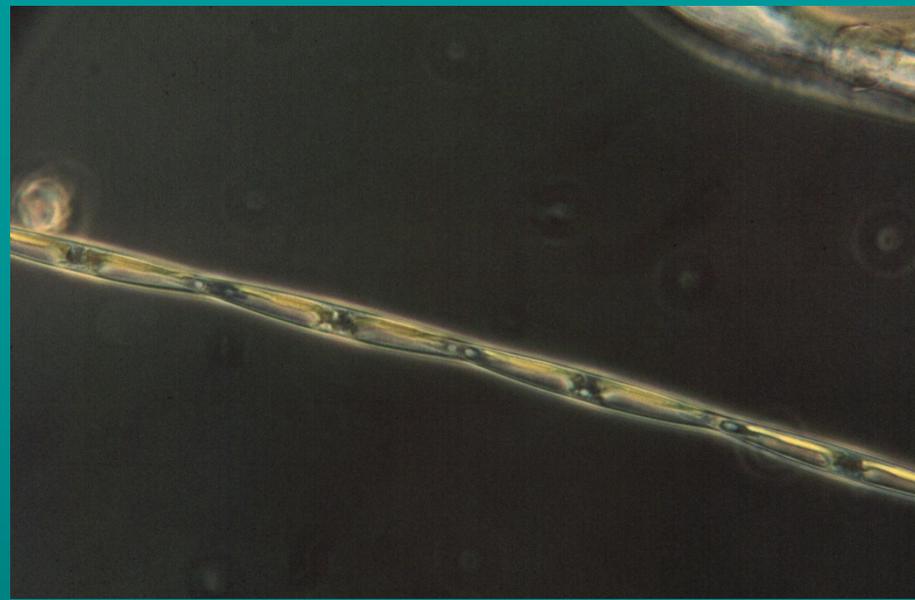
Domoic Acid

Neurotoxin produced by a no. of algae, especially *Pseudonizschia australis*

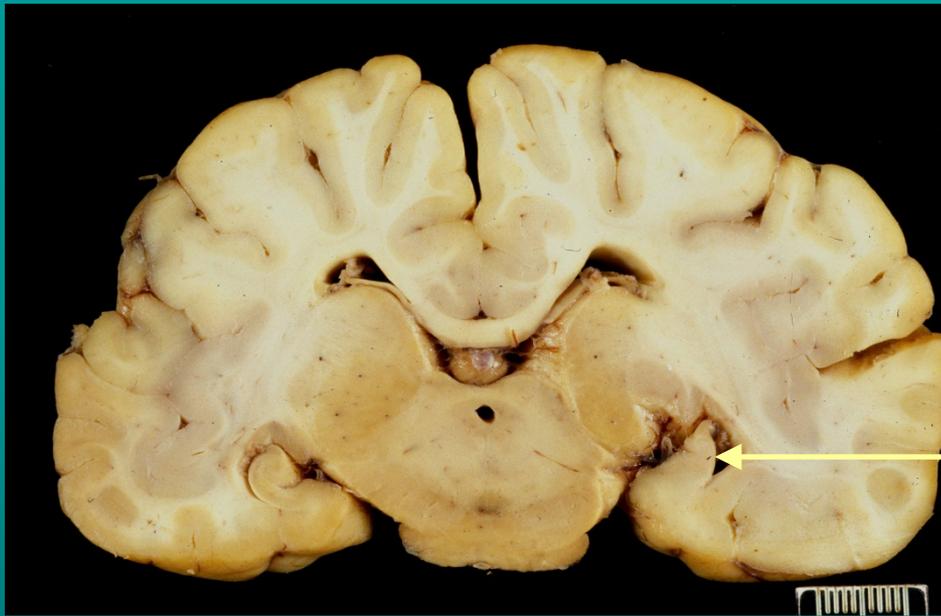
Binds to glutamate receptors in the CA3, CA4 regions of the hippocampus, causing neurological signs

First identified when about 100 humans were poisoned, 3 died, in eastern Canada in 1987

Domoic acid toxicity (Amnesic shellfish poisoning)



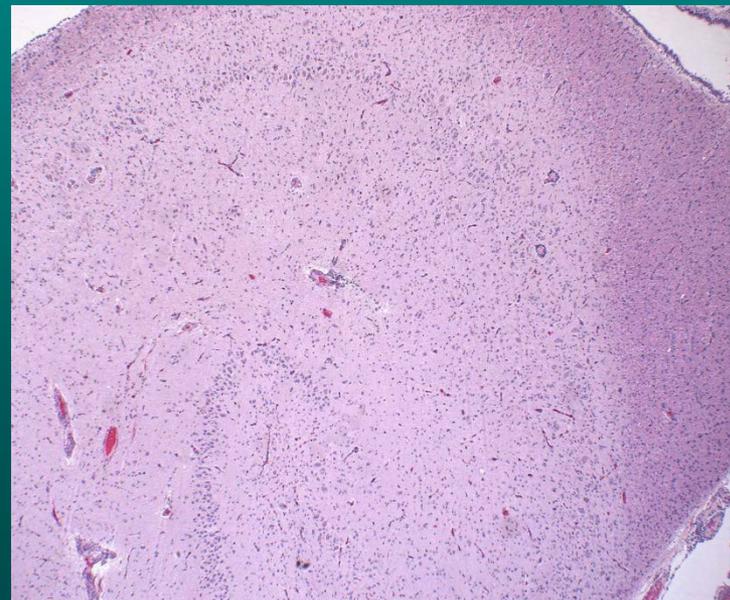
- Strandings of marine mammals associated with domoic acid in California have been increasingly reported since 1998
- Clinical signs associated with mortality events include seizures, disorientation, ataxia, blindness, coma, and death in California sea lions
- Apparently recovered animals are released after treatment



**hippocampal
atrophy**



Normal hippocampus



Gliosis and neuronal loss

Diagnosis of domoic acid poisoning in California sea lions

Clinical signs

Histopathology

Domoic acid in body fluids

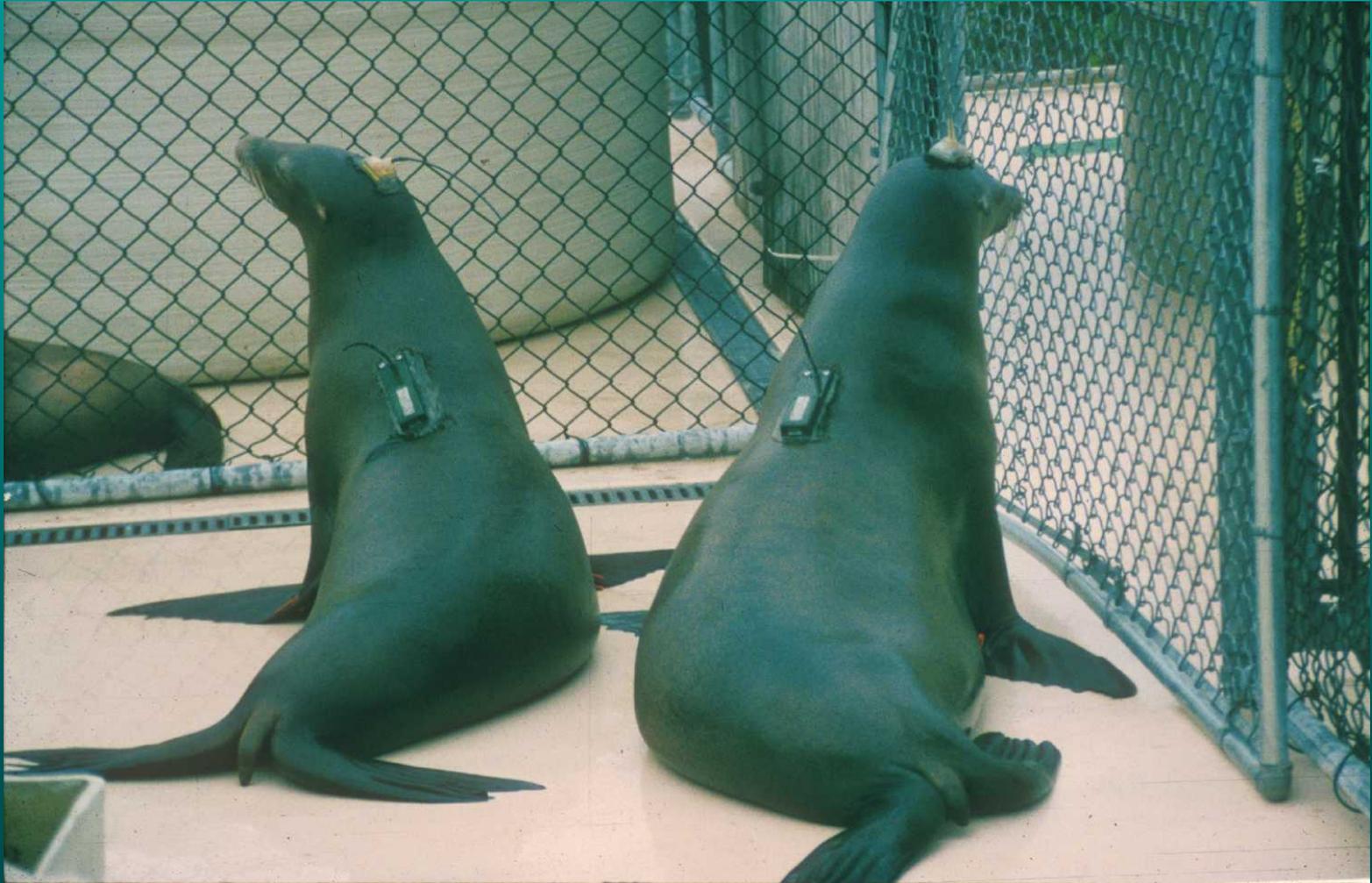
urine most reliable

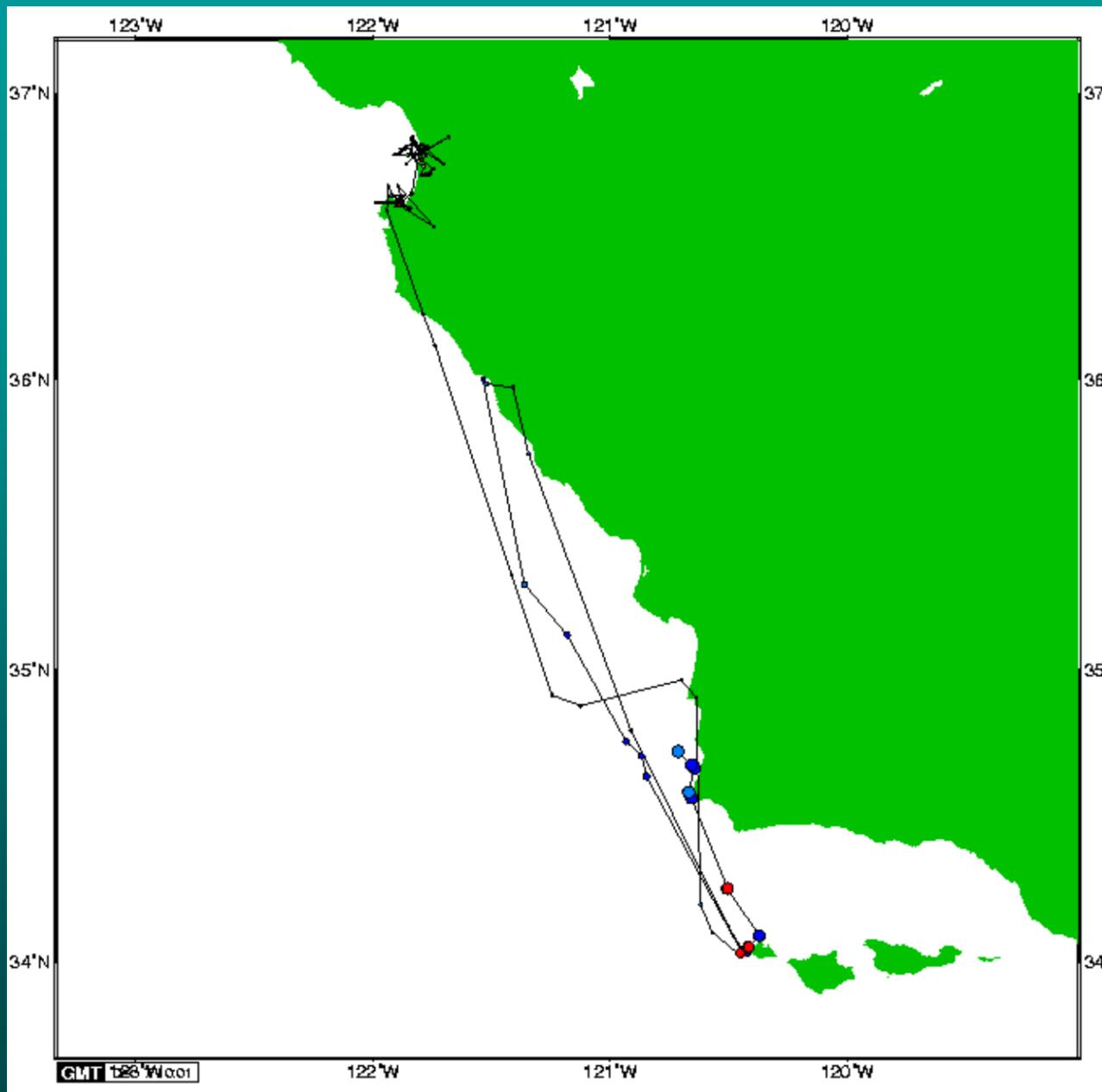
Anchovies in feces

***Pseudo-nitzschia australis* in anchovies**

***Pseudo-nitzschia australis* blooms**

Long-term prognosis?





Tick CSL 3719

- Restranded April 21, 2002
- Aborted still-born pup
- Seizures, ataxia, obtunded
- Treated with domoic acid protocol
 - Lorazepam and 0.03 – 0.04 mg/kg if seizures
 - Phenobarbital 4 mg/kg PO/IM BID x 2 days
 - Phenobarbital 2 mg/kg PO/IM additional 5 days
 - If seizures, increase phenobarbital by 2 mg/kg for 5 days and the taper by 2 mg/kg every 5 days
 - Release if no seizures and normal neurologic evaluation 7 days after last phenobarbital
 - Released 4 weeks after admit
- Now what is long-term prognosis and what are population level effects?

Abortion, still birth, premature parturition





1998 70 sea lions – first diagnosis

2000 206 sea lions, sea otters, gray whale

2001 67 sea lions

2002 450 sea lions

120 common dolphins

3 harbor seals

3 harbor porpoise

sea otters

2003, 2004 multiple species

**And it AIN'T STOPPING
more years with outbreaks
than not**



Long-term effects of domoic acid toxicity



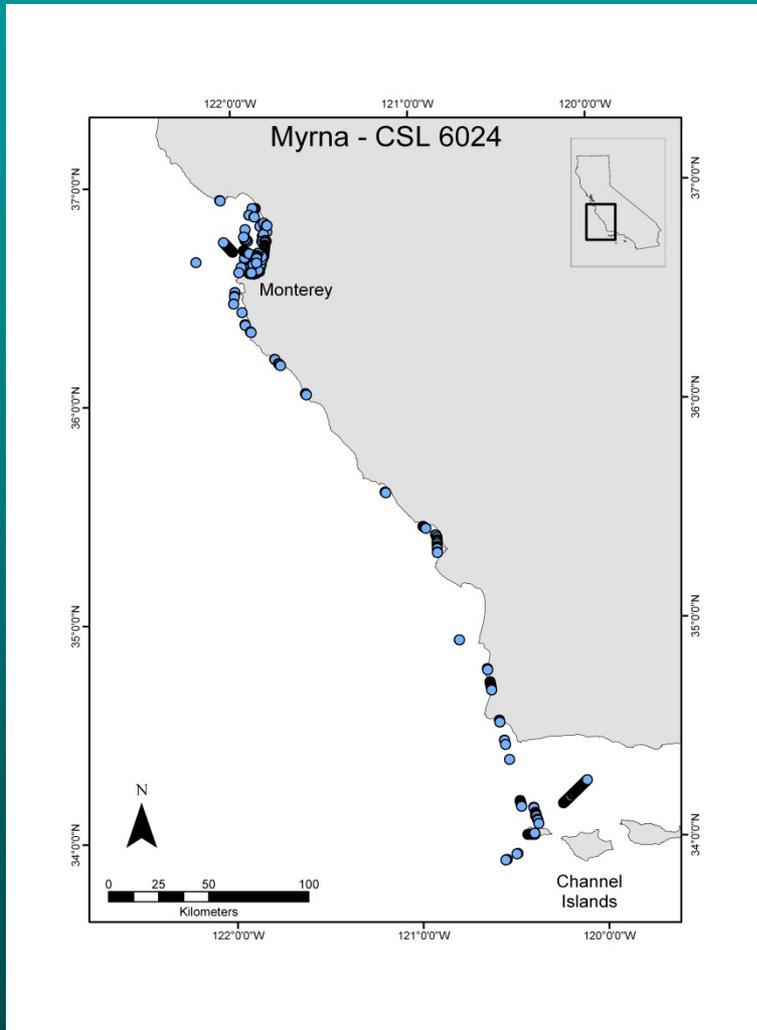
Goals

1. Identify animals with long-term effects
2. Evaluate post-release movements and dive behavior
3. Develop criteria to evaluate prognosis for post-release survival of affected animals

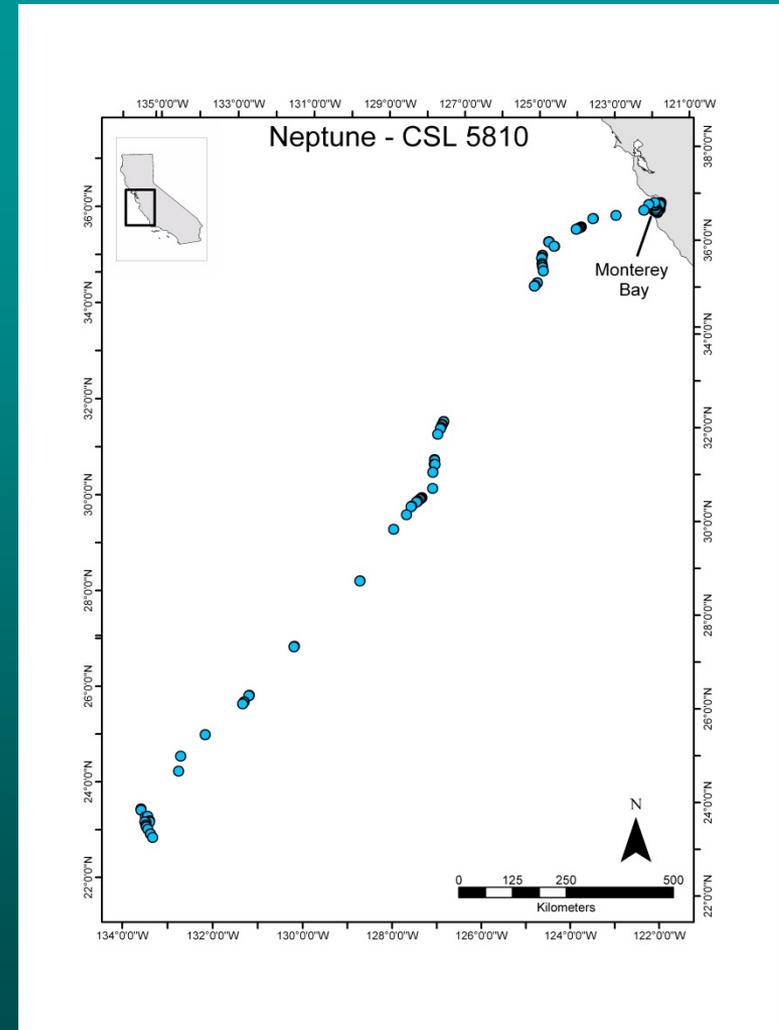
Sea lions suspected of having long-term effects

- Exhibit neurologic signs typical of domoic acid toxicity yet strand during a time of no known domoic acid producing algal blooms
- Re-strand after initial treatment for domoic acid toxicity
- Continue to exhibit neurologic signs after multiple courses of anti-convulsant therapy

Post-release monitoring



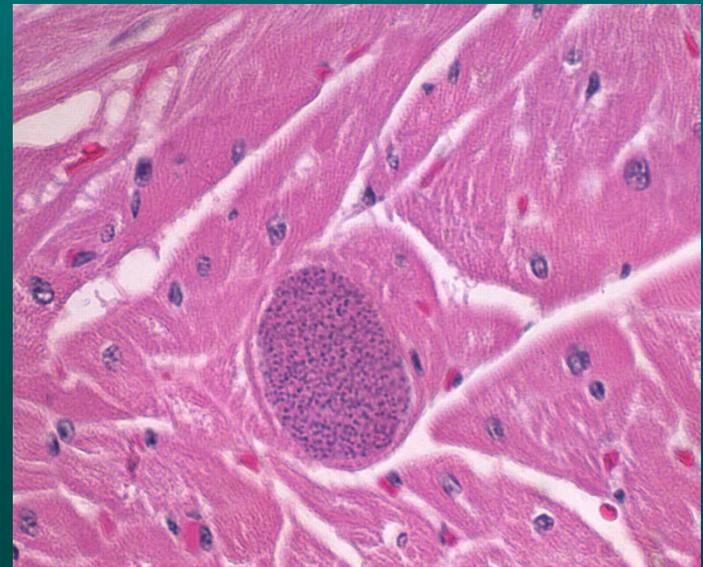
Normal movement



Abnormal movement

Confounding findings

- CSL 6018
 - Gunshot on radiograph
 - No intracranial hemorrhage
 - MRI findings consistent with domoic acid toxicity
- CSL 5531
 - *Toxoplasma* titer = 1: 81, 920
 - MRI findings consistent with domoic acid toxicity
 - Histopathology consistent with domoic acid toxicity
 - Protozoal cysts found in a variety of tissues other than brain



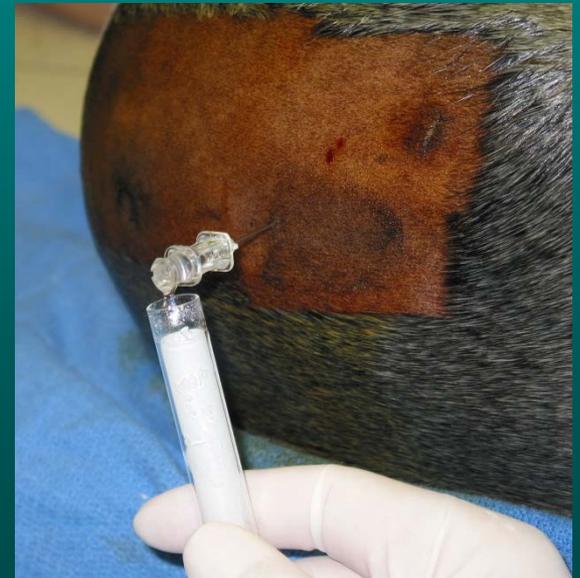
Long-term effects of domoic acid toxicity

- Suspected since 2000
 - Approximately 150 animals
- Sea lions may display abnormal behavior
 - Repeated stranding often in unusual locations
 - Abnormal aggression
 - Abnormal tameness
 - Stereotypy
 - Flipper chewing
 - Ingestion of foreign bodies
 - Pacing
- Animals may not show obvious seizure activity



Differentiating other causes of neurologic signs in stranded sea lions

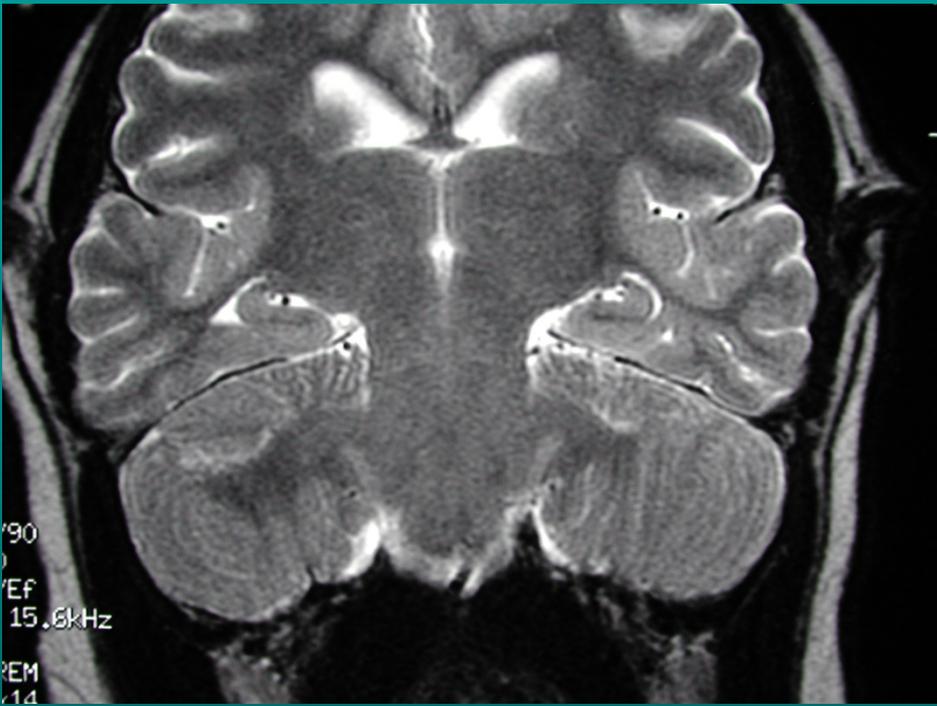
- Complete blood counts and serum biochemistry analysis
- Serology for *Toxoplasma* sp., *Neospora* sp., and *Sarcocystis* sp.
- Radiographs to rule out trauma
- Cerebrospinal fluid evaluation
- Magnetic resonance imaging (MRI)



Magnetic resonance imaging

- Brain is sliced coronally through the temporal lobes using the same orientation as the pathologists
- Sectioning is orthogonal to the long axis of the temporal horn (ventricle of the temporal lobe)





Normal



Unilateral hippocampal atrophy and temporal horn enlargement

MRI Results

- Most common MRI findings include unilateral (n=8) and bilateral (n=4) hippocampal atrophy found in all 12 animals
- MRI lesions in 3 animals (confirmed by histopathology) suggested other causes of seizures
 - Cerebritis
 - Subarachnoid hemorrhage



Limitations of MRI

- Relationship of severity of hippocampal atrophy to brain function in the sea lion is not well understood
- Subtle changes may be missed
- Malfunction at the cellular level cannot be addressed
- Labor intensive and cost prohibitive

Post-release movement vs. severity of MRI lesions

Normal “behavior”

- 2 animals with mild changes
- 1 animal with moderate lesions
- 1 animal with severe changes

Abnormal “behavior”

- 1 animal with mild changes
- 2 animals with moderate changes

Electroencephalography

- Science relating to the electrical activity of the brain
- Used in a variety of other species to determine brain activity
- Potential to demonstrate functional changes
- Potential to show sub-clinical seizure activity

Electroencephalography

- Background activity
 - Muscle tremors due to sedative agents
 - Movement
- Paroxysm
 - phenomenon with abrupt onset
 - rapid attainment of a maximum
 - sudden termination
 - distinguished from background activity
 - used to refer to epileptiform patterns and seizure patterns

EEG Terminology

Input terminal 1 - the input terminal of the differential EEG amplifier at which negativity, relative to the other input terminal, produces an upward trace deflection.

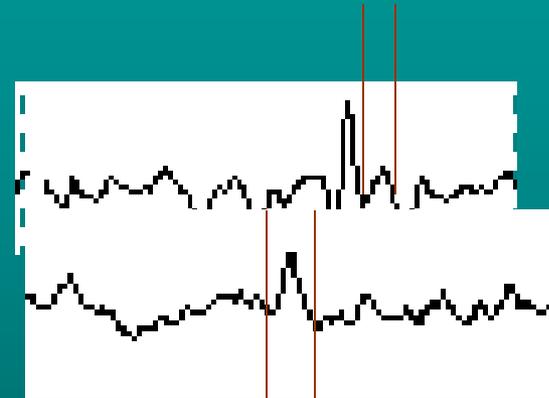
Input terminal 2 - the input terminal of the differential EEG amplifier at which negativity, relative to the other input terminal, produces a downward trace deflection.

Derivation – 1) the process of recording from a pair of electrodes in an EEG channel, 2) the EEG record obtained by this process.

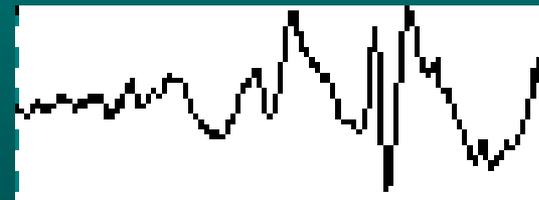
Montage - the particular arrangement by which a number of derivations are displayed simultaneously in an EEG record.

Paroxysmal Events

- Spikes
 - duration < 70 msec
- Sharp Waves
 - between 70 - 200 msec



- Spike-and-Wave
- can occur singly or in complexes



Sea lion encephalography

- 24 animals suspected of surviving previous domoic acid poisoning
 - 22 animals had received anticonvulsant therapy
- 6 control animals
- Sedated with medetomidine (0.07 mg/kg) IM
 - butorphanol (0.1 mg/kg IM) was also given but was discontinued due to muscle fasciculations

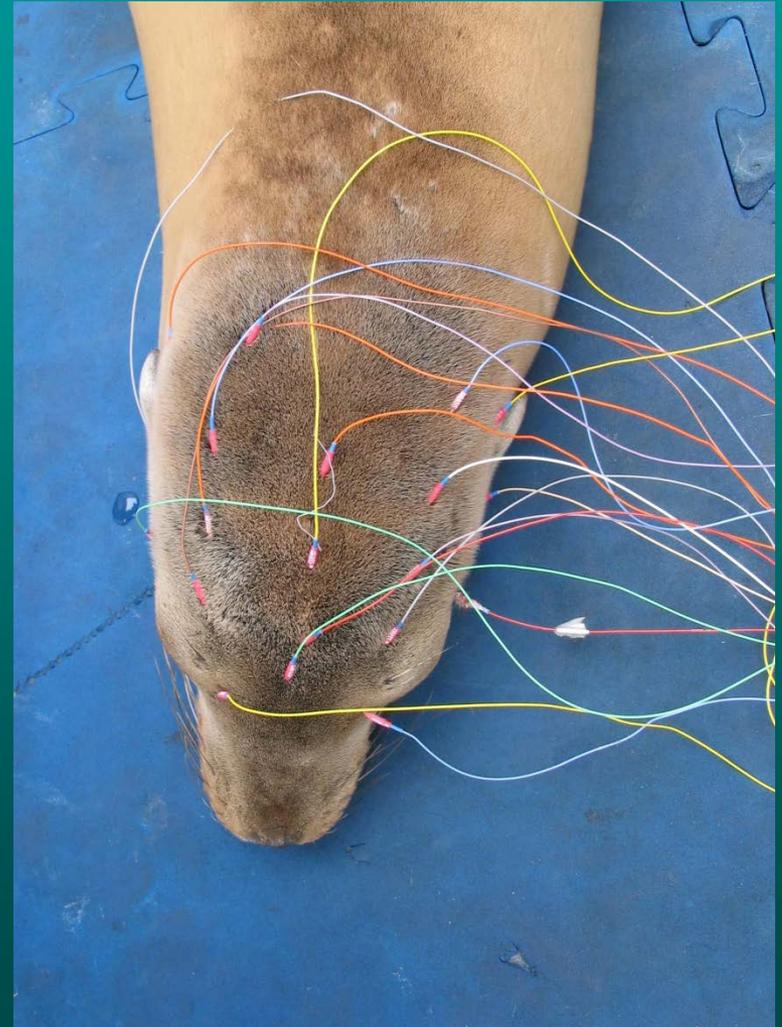
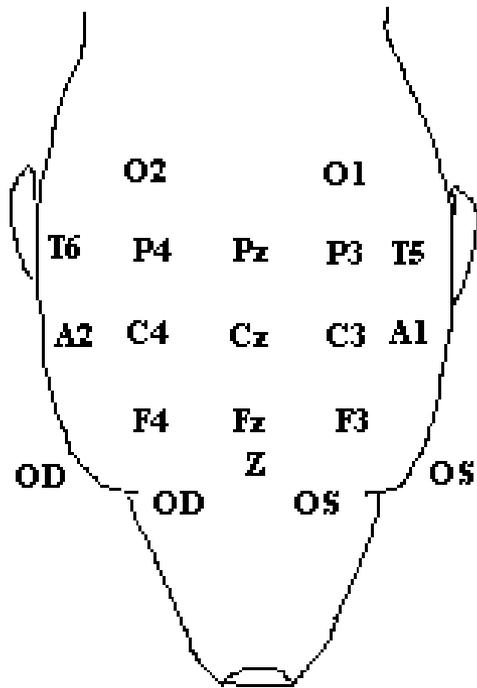


Sea lion encephalography

- Electrodes
 - 27 gauge needle electrodes
 - 15 EEG
 - 4 EOG
 - 2 EKG
 - Ground
- Double banana montage
 - based on a standard canine system
- Minimum recording duration 10 minutes
 - 4 animals had repeat EEGs at least one week after the initial recording took place

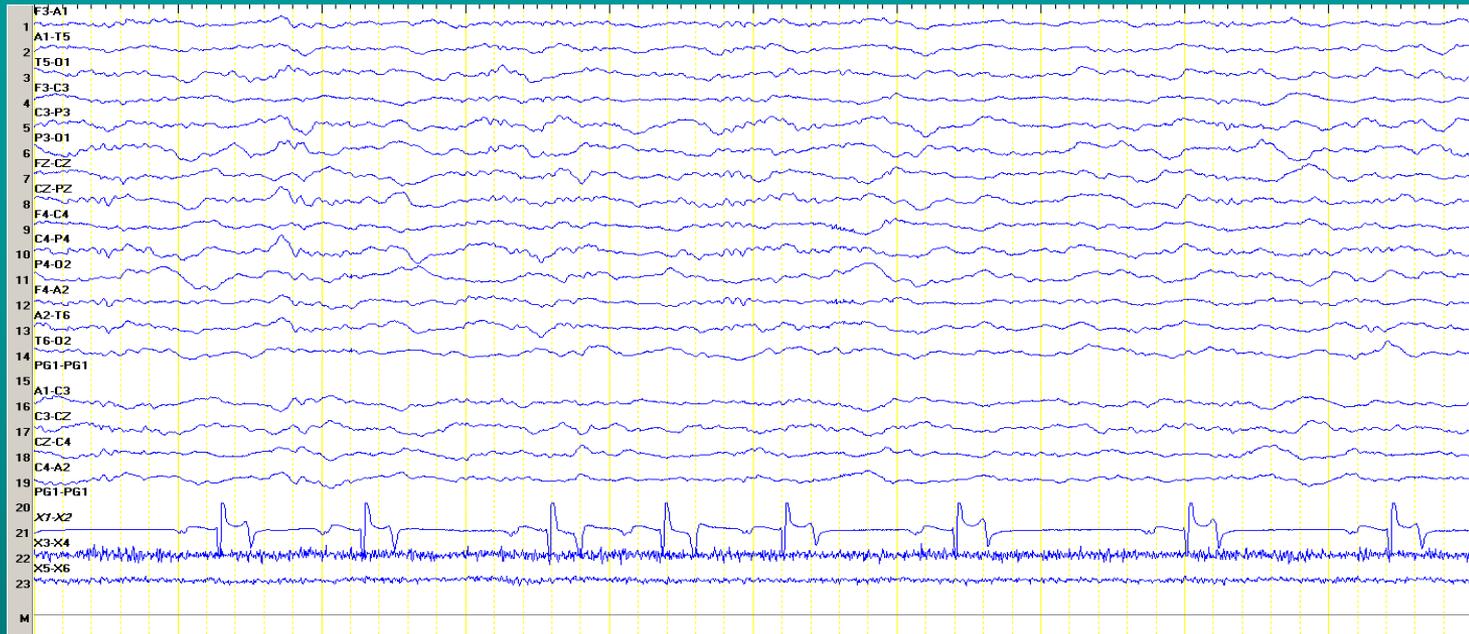


Montage

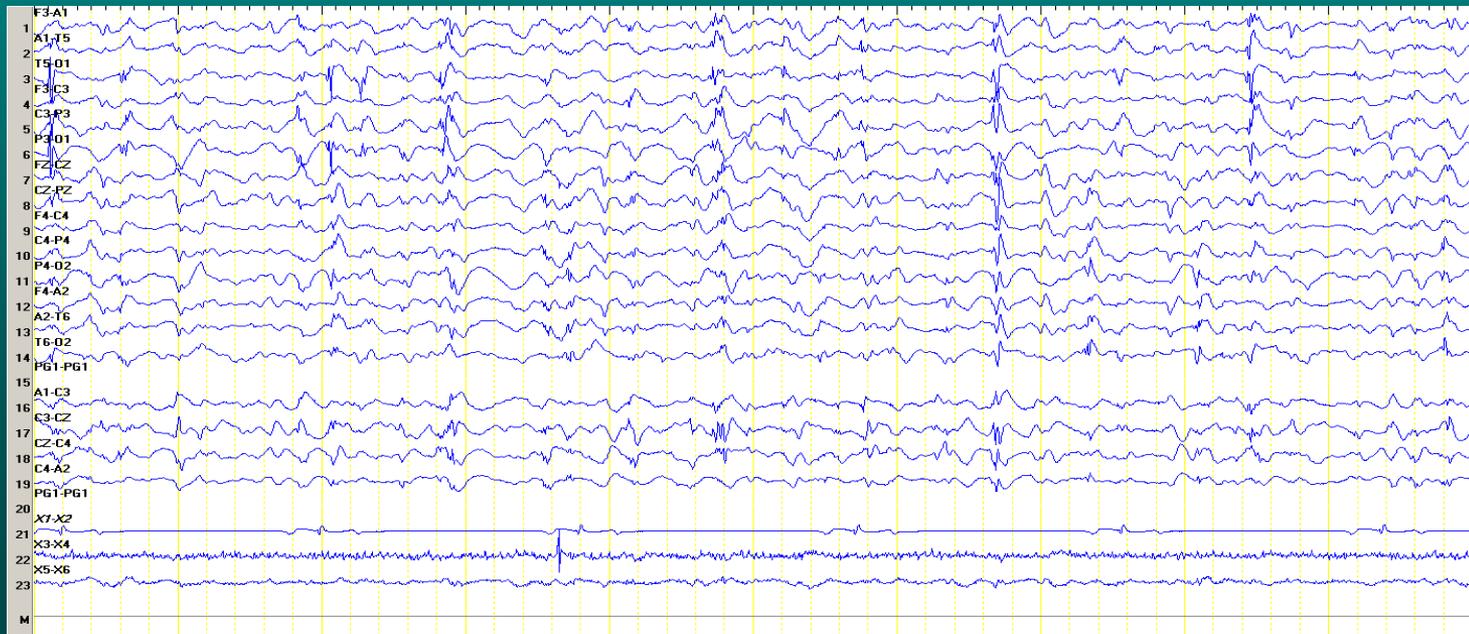


EEG

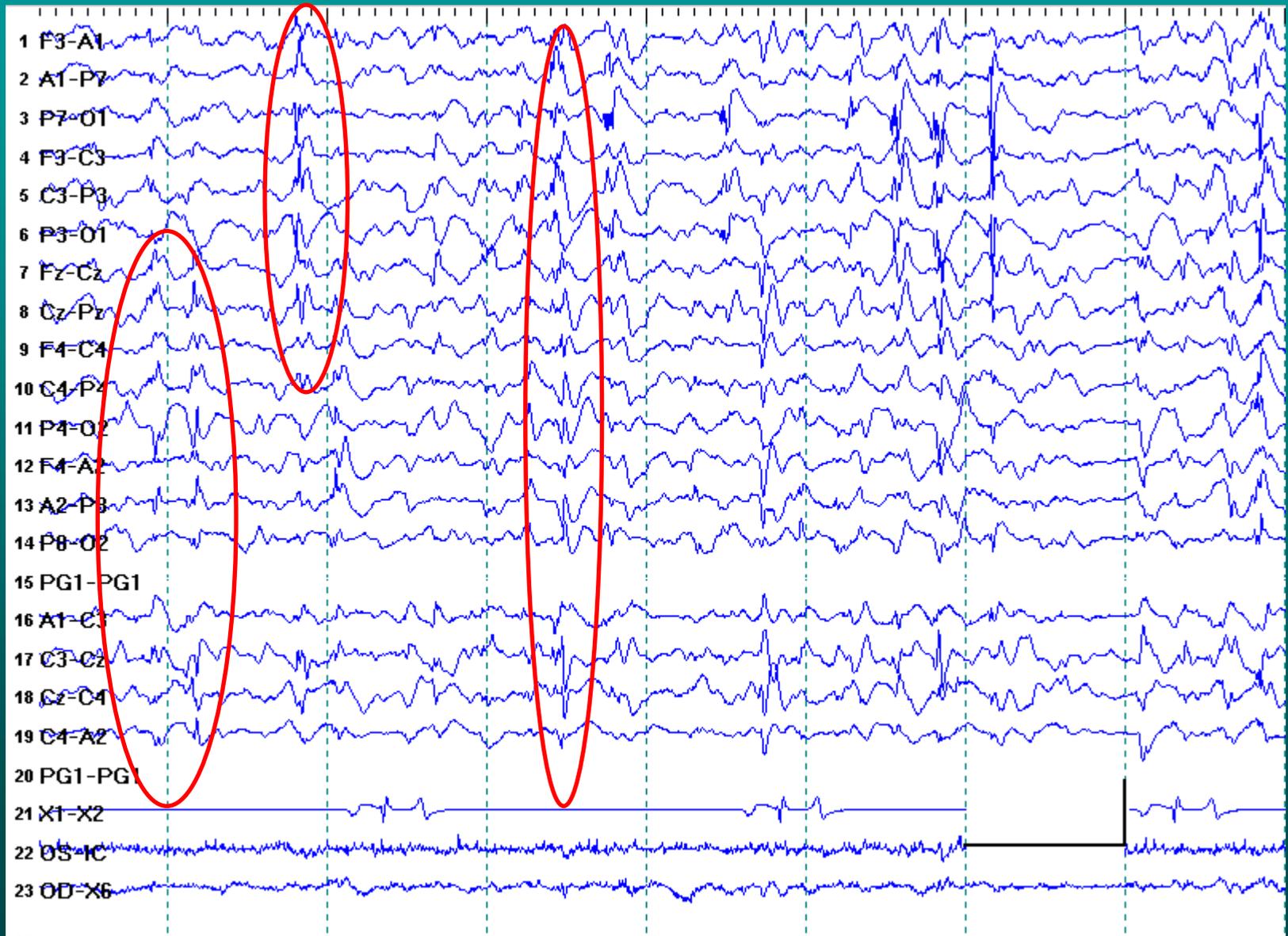
Normal



Epileptiform pattern

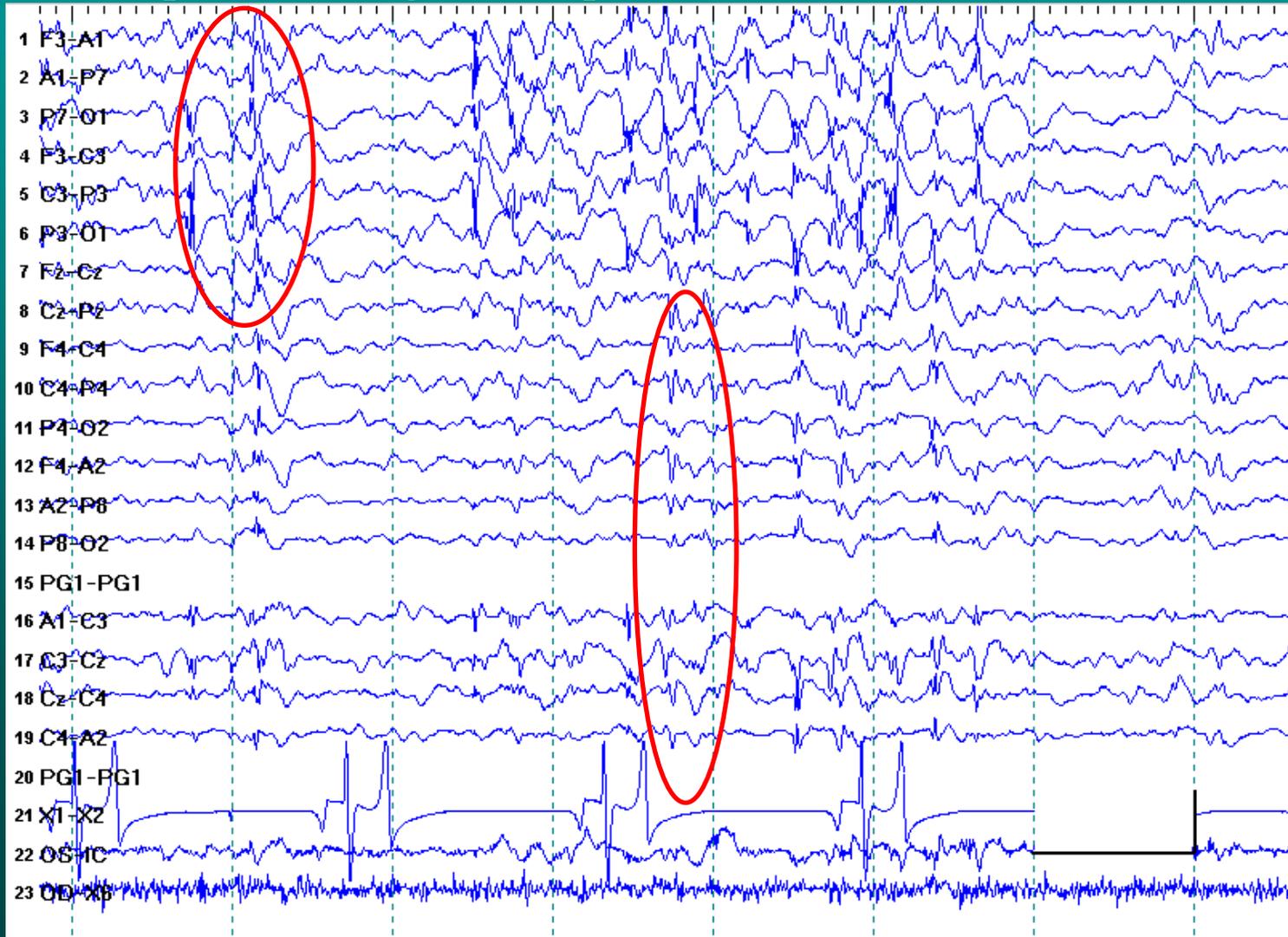


Multifocal spikes and sharp waves

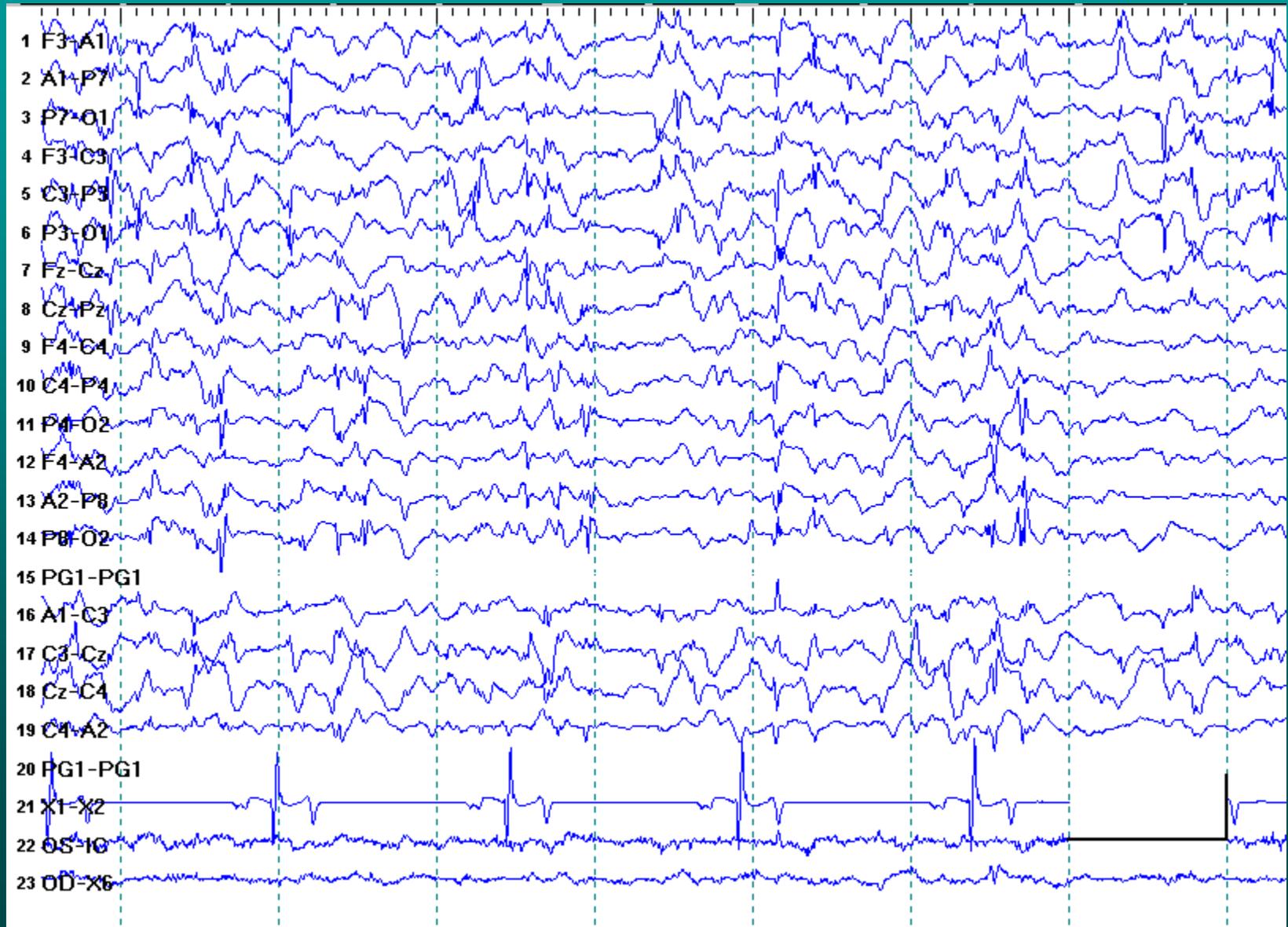


Lateralized events

- spike-and-wave discharges in left hemisphere
- spikes and sharp waves in right hemisphere



A little bit of everything



Results and conclusions

- Epileptiform activity, consisting of spikes, sharp waves and spike-and-waves, was noted in EEGs obtained from all 24 sea lions suspected of previous exposure to domoic acid
- These events were generalized and multifocal in most patients, though in a few cases, were more prominent in one hemisphere
- No such changes were observed in the EEGs recorded from control animals.
- These findings are supportive of a diagnosis of secondary epilepsy as the result of domoic acid exposure in these California sea lions.

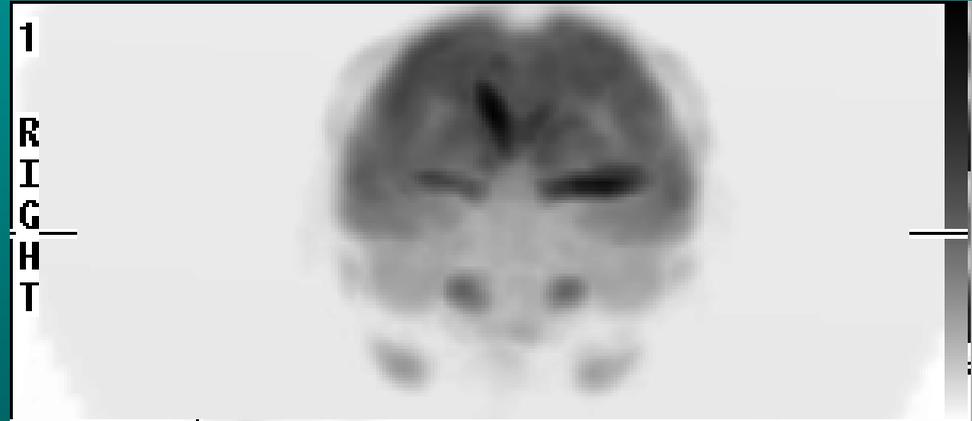
Where do we go from here?

The variable post-release success of animals diagnosed with long-term effects of domoic acid toxicity suggests that diagnosis of the severity of lesions must be improved in order to better evaluate the prognosis for affected California sea lions

Other diagnostic modalities?

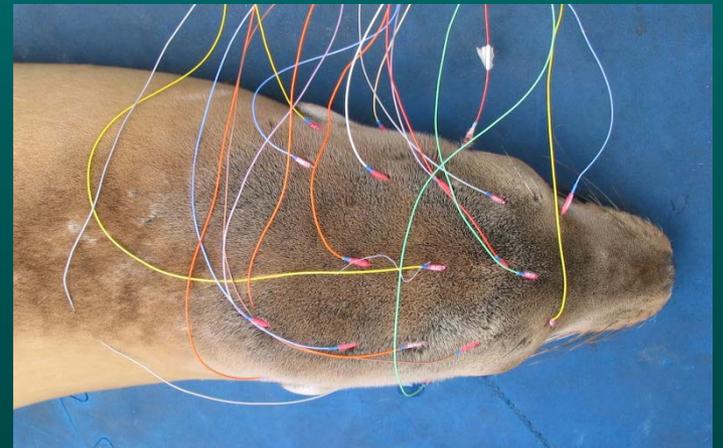
PET scan

EEG



Other prognostic indicators?

Assess memory and cognitive function



PET Scan



Acknowledgements

- Staff and volunteers of The Marine Mammal Center
- NOAA Fisheries Prescott Grant Program
- Arthur and Elena Court Nature Conservancy
- Page Evans Veterinary Fellowship
- Pat Conrad for running protozoal serology



