The basis for breathing and autonomic disturbances in RETT Syndrome
Large catalogue of breathing abnormalities have been described by Allison Kerr.
Breathing is only one of several disturbances that are all somehow interconnected and relate to disturbances in the brain.
Our brain is persistently active
Our brain is persistently active

Awake brain state
Persistent activity requires close coordination between brain, lungs and heart.

Persistent activity requires 95% of metabolic energy.
Breathing is an important timing mechanism in the brain. Brain provides important timing clues to breathing. Breathing is an important driver of arousal.
Inspiration is driven by the preBötzinger complex.

Cardiovagal neurons inhibit the heart.
The Pre-Bötzinger complex & Nucleus ambiguus — preBötC NA
Breathing and heart overlap functionally

Inspiration  Expiration

Airflow

Heart rate

Breathing and heart functionally overlap: “diving response”
Cardiorespiratory Coupling is disturbed in “breath-holds” of Rett Syndrome children
Cardiorespiratory coupling is disturbed in Rett Syndrome: NOT “Diving response”
Many treatments have been tried to control breathing disturbances with variable “success”.

These abnormalities typically start after the children turn two years.
There is large individual variability in the response to treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Why?</th>
<th>How?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiepilepsy drugs</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Serotonin agonist</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Benzodiazepine</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SSRIs</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Use of equipment</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Oxygen</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Rebreathing apparatus</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>CPAP/BIPAP</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Compression vest</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

From: Mackay et al. 2017  
Journal of Neurodevelopmental Disorders 9:15
Disease progression is not linear and many factors are interacting.

One driver of disease progression could be the Breath-holds.

<table>
<thead>
<tr>
<th>Rapid regression</th>
<th>Autistic features</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Loss of hand skills, speech, and social interaction</td>
</tr>
<tr>
<td></td>
<td>Hand stereotypies</td>
</tr>
<tr>
<td></td>
<td>Mental retardation</td>
</tr>
<tr>
<td></td>
<td>Motor abnormalities</td>
</tr>
<tr>
<td></td>
<td>Seizures</td>
</tr>
<tr>
<td>Respiratory abnormalities</td>
<td></td>
</tr>
<tr>
<td>Stationary stage</td>
<td>Scoliosis</td>
</tr>
<tr>
<td></td>
<td>Autonomic dysfunction</td>
</tr>
<tr>
<td></td>
<td>Anxiety</td>
</tr>
<tr>
<td>Late motor deterioration</td>
<td>Decrease/loss of mobility</td>
</tr>
<tr>
<td>Parkinsonian features</td>
<td></td>
</tr>
</tbody>
</table>
Why?  

Breath-holds cause Hypoxia & oxidative stress

How?

Oxidative Stress

Hypoxic Stress

One Breath-hold
Hypoxic Stress

Why?

Oxidative Stress

How?

Breath holds cause intermittent hypoxia

Hypoxic Stress
Breath-holds cause intermittent hypoxia

Why?  How?

Oxidative Stress is exaggerated by mitochondrial dysfunction (Eubanks)

Hypoxic Stress
Intermittent hypoxia and in particular Oxidative stress affects the network that couples breathing and the heart.
Hypoxia causes oxidative stress

EXPERIMENTAL MODEL

Fred Garcia

Oxidative Stress

Lipid Peroxygeneration
Hypoxia causes oxidative stress

RETT SYNDROME

EXPERIMENTAL MODEL

Lipid Peroxygenation


$\text{H}_2\text{O}_2$ decreases cardiac vagal activity & blunts respiratory modulation of cardiac tone.

**EXPERIMENTAL MODEL**

![Control graph with peak highlights](image-url)

2 sec
H$_2$O$_2$ decreases cardiac vagal activity & blunts respiratory modulation of cardiac tone.

EXPERIMENTAL MODEL

RET'T SYNDROME

Weese-Mayer et al. 2006
We performed a new analysis of various cardiorespiratory measures

Carroll et al. in prep.
Respiratory Sinus Arrhythmia has a circadian dependence

Rett – blue
Control - red

Carroll et al. in prep.
Heart rate has also a circadian effect

Carroll et al. in prep.
Geno-phenotype relationships

Rett – blue
Control - red
The pre-Bötzinger complex & Nucleus ambiguus -

- Hypoxic Stress
- Oxidative Stress

Breath-hold

Nucleus ambiguus

Vagus pre-BötzC
The Pre-Bötzinger complex & Nucleus ambiguus

Oxidative Stress
Hypoxic Stress

Breath-hold

heart rate

Nucleus ambiguus

Vagus pre-BötzC
Intermittent hypoxia also alters the preBötzinger complex.
Chronic Intermittent Hypoxia (CIH) decreases intrinsic excitability in inspiratory neurons

We record from neurons in preBötC

**nauhoitamme preBötzinger-kompleksin neuroneista**

Garcia et al. 2017, Frontiers in Physiology
Garcia et al. 2016, Frontiers in Neuroscience
The respiratory network becomes de-synchronized following CIH
The respiratory network becomes de-synchronized following CIH

MORE INCOMPLETE SYNCHRONIZATION = breathing becomes irregular

Garcia et al. 2016
Breathing abnormalities in Rett Syndrome show large individual variation.

**EXPERIMENTAL MODEL**

Garcia et al. 2016

**RETTE SYNDROME**

Weese-Mayer et al. 2006

Viemari et al. 2005

Control

Intermittent hypoxia

Mecp-/- mouse
Breathing abnormalities in Rett Syndrome show large individual variation.
Not only breathing irregularities, but also the occurrence of apneas shows individual variability.

Rohdin et al. 2007
Pediatr Neurol
37:338-344.
There are also daily variations within an individual patient.

Why?

Rohdin et al. 2007
Pediatr Neurol
37:338-344.

Day-by-day variations in one patient
Another major contributor to individual variability are modulators.
Breathing depends on many neuromodulators

Neuromodulation is dysregulated in Rett Syndrome

Ramirez & Doi,
Resp Physiol Neurobiol. 2008
NE levels are reduced in Rett Syndrome and its mouse model.

Viemari et al. 2005, J. Neuroscience

Can neuromodulation be used to treat Rett Syndrome?
Can neuromodulation be used to treat Rett Syndrome?

But the situation is not so simple!

A word of caution

Viemari et al. 2005
J. Neuroscience
Rett Syndrome is associated with intermittent hypoxia. Intermittent hypoxia affects the response to neuromodulators.

We can experimentally apply intermittent hypoxia.
Norepinephrine with Intermittent hypoxia causes irregularities
Stimulating locus ceruleus

Norepinephrine release combined with intermittent hypoxia causes irregularities

Zanella et al. 2013, J. Neuroscience

May explain why desipramine trial was not successful
May explain why desipramine trial was not successful
This “paradoxical” neuromodulator response may explain the state dependency of Rett Syndrome.

Breathing disturbances are state dependent.

May also explain why breathing is disturbed during the day.
If not norepinephrine –

Can we treat Rett Syndrome by targeting oxidative stress?

Oxidative Stress is exaggerated by mitochondrial dysfunction (Eubanks)
The effect of intermittent hypoxia can be prevented by ROS scavengers.

Naïve preBötC

Int. preBötC

CIH

ROS scavenger

Int. preBötC

Int. preBötC

Naïve preBötC

The effect of intermittent hypoxia can be prevented by ROS scavengers.
The Pre-Bötzinger complex

What are there clinical implications?

EXPERIMENTAL MODEL

Garcia et al. 2016

ROS scavenger

Control

Intermittent hypoxia

Intermittent hypoxia

ROS scavenger

“Rett mouse”

RETT SYNDROME

Weese-Mayer et al. 2006

Viemari et al. 2005

ROS scavenger
What are there clinical implications?

Children with breathing irregularities may respond to anti-oxidant treatment.

Anti-oxidant treatment may prevent development of breathing irregularities.

But this treatment will unlikely abolish the breath-holds.

Antioxidants may prevent development of breathing irregularities, cardiorespiratory uncoupling, cognitive decline.

Breathing irregularities in Rett Syndrome: Defining the breathing abnormalities in children with breathing irregularities may respond to anti-oxidant treatment. Antioxidants may prevent development of breathing irregularities, cardiorespiratory uncoupling, cognitive decline.
What are breath holds and the mechanisms leading to breath-holds?

Breath-holds persist after anti-oxidant treatment

Janc et al. 2016
Frontiers in Cellular Neuroscience

Breath-holds persist after anti-oxidant treatment
Defining the **breathing** abnormalities in Rett Syndrome

- **preBötzinger complex**
- **Postinspiratory Complex** (PiCo)
- **Inspiration**
- **Postinspiration**
- **Active Expiration**
- **High metabolic demand**
- **Parafacial Resp. Group (pFRG)**

Three **excitatory microcircuits**
Breath-holds in Mecp2 mutant mouse are characterized by overactive post-I and active Expiration.

Abdala AP, Dutschmann M, Bissonnette JM, Paton JF.
Anatomical location of PiCo and pFRG
Overlap between pFRG and presympathetic neurons
Pons seems to play a role in overactivating post-I and active E

Control from pons

Abdala AP, Dutschmann M, Bissonnette JM, Paton JF.
Activation of 5-HT1a agonist in pons reduces breath-holds in mouse model

*Sarizotan – Clinical trial*

Cortex seems to play a role in overactivating post-I and active E

Control from Cortex

Control from pons


Abdala AP, Dutschmann M, Bissonnette JM, Paton JF.

N-Methyl-D-Aspartate Receptors, Ketamine, and Rett Syndrome: Something Special on the Road to Treatments?

David M.Katz a
Frank S.Menniti b
Robert J.Mather c

Biological Psychiatry
Volume 79, Issue 9, 1 May 2016, Pages 710-712
Brain activity mapping in MeCP2 mutant mice reveals functional deficits in forebrain circuits, including key nodes in the default mode network, that are reversed with ketamine treatment.

Kron M¹, Howell CJ, Adams IT, Ransbottom M, Christian D, Ogier M, Katz DM.

*Ketamine – Clinical trial*
Why is Rett syndrome so complicated to treat?

- Disease progression is not linear and differentiated.
- Disease progression is personal.
- Disease progression depends on neuromodulatory milieu.

Breathing disturbances are state dependent.

For the treatment of Rett Syndrome, it will be important to consider these homeostatic mechanisms.
We performed a new analysis of various cardiorespiratory measures

Carroll et al. in prep.
Respiratory Sinus Arrhythmia has a circadian dependence

Carroll et al. in prep.
Heart rate has also a circadian effect

Carroll et al. in prep.
Geno-phenotype relationships