Impact of Sleep Deprivation Stress in Promoting Sensitization of the Trigeminal System
Functions of Sleep

- Fatigue reversal
  - allows individual to recover and reenergize

- Biochemical refreshment
  - promotes synaptic efficiency, protein synthesis, neurogenesis, metabolic restoration

- Immune function
  - reset or protection

- Memory
  - memory consolidation, facilitates encoding new information

- Psychologic well-being
  - lack of sleep: risk of mood alteration to depression
As the night progresses, the length of REM stages increases and the length of time spent in deep sleep decreases. By morning, nearly all sleep is stage one, two or REM.

Typical cycle: 7 1/2 - 9 hrs
Sleep Deprivation: Major Epidemic

- Significant impact on public health
- 20%-60% hospitalized patients
- 20% general population
- Incidence and prevalence increasing in youth and young adults
- Triggers:
  - mood alteration
  - sociability dysfunction
  - complaints of bodily pain
Sleep Quality/Quantity and Pain – Vicious Cycle

- Bad night’s sleep enhances pain – but pain disrupts sleep creating vicious cycle
- Sleep loss increases hyperalgesia and spontaneous pain
- Pain perception - dependent on activity of ascending (stimulatory) and descending (inhibitory) pathways
- Potential mechanisms contributing to sleep loss-induced pain enhancement:
  - opioid, monoaminergic, HPA, melatonin, and immune (inflammatory) systems
- Sleep loss – increased levels of proinflammatory cytokines (IL-1β, IL-6, TNF-α) – potent pain inducing and facilitating factors
TMD (Migraine) - Sleep - Pain

- TMD patients exhibit enhanced responses to painful stimuli
- Genetic polymorphorphisms predict development of new onset TMD
- Peripheral and central contributions early - but as progresses, changes in central pain processing (central sensitivity syndromes)
- Evidence that sleep disturbance – factor that directly contributes to central sensitization and pain amplification
- 77% of orofacial pain patients report reduced sleep quality and quantity of sleep
- 75% ICSD self-reported sleep bruxism; 17% met RDC PSG criteria for active
- 43% diagnosed with 2 or more sleep disturbances
  - primary insomnia (36%), obstructive sleep apnea (28.4%)

Smith et al., *SLEEP*, 2009
REM Sleep Deprivation Suppresses Phosphatase Levels in Trigeminal Neurons and Glia

Promotion of Peripheral and Central Sensitization
TMD Pathology - Increased Expression of Pro-Inflammatory/Nociceptive Signaling Molecules and Increased Neuron-Satellite Glial Cell Communication

Tissue Injury or Sleep Deprivation

IL-1β, IL-6, TNF
PG, ATP, NO

Activation/Sensitization of primary afferents

TMJ Muscle

Neuropeptide Release

Sleep deprivation – increased levels of proinflammatory cytokines (IL-1β, IL-6, TNF-α)

Neuronal Cell Body

Satellite Glia

Trigeminal Nucleus

Activation of Second Order Neurons Glia Cells

Pain

Trigeminal Neuron
Normal Neuronal or Glial Cell at Homeostasis

Kinases
Present in Neurons and Glial Cells
Increase Expression of Proteins and Molecules Involved in Inflammation and Nociception

Phosphatases
Present in Neurons and Glial Cells
Decrease Expression of Proteins and Molecules Involved in Inflammation and Nociception

Inflammatory Signaling Molecules, Gap Junctions and Ion Channels
Phosphatase Activity
Neuronal or Glial Cell During Painful Phase

Kinases
Increase Expression of Proteins and Molecules Involved in Inflammation and Nociception

Phosphatases
Decrease Expression of Proteins and Molecules Involved in Inflammation and Nociception
Normal Neuronal or Glial Cell Restorative Phase

Kinases

*Increase Expression of Proteins and Molecules Involved in Inflammation and Nociception*

Phosphatases

*Decrease Expression of Proteins and Molecules Involved in Inflammation and Nociception*
REM Sleep Deprivation After TMJ Inflammation Leads to Chronic Increased Levels of Nociceptive Proteins in Trigeminal Neurons and Glia

ERK = MAP Kinase
PKA = Protein Kinase A
P2X3 = ATP Ion Channel

RSD = REM Sleep Deprivation; CFA = complete Freund’s adjuvant; heat killed bacteria - inflammatory
REM Sleep Deprivation After TMJ Inflammation Leads to Prolonged Decreased Levels of Phosphatases in Trigeminal Neurons and Glia

RSD = REM Sleep Deprivation

MKP-1 and MKP-3
Anti-inflammatory proteins

CON
MKP-1

CFA d21
MKP-1

CFA d21
MKP-1 + RSD

CON
MKP-3

CFA d21
MKP-3

CFA d21
MKP-3 + RSD
REM Sleep Deprivation After TMJ Inflammation Leads to Chronic Increased Levels of Inflammatory Proteins In STN

p38 - inflammatory protein; OX-42 – biomarker of activated microglia
REM Sleep Deprivation After TMJ Inflammation Leads to Chronic Decreased Levels of MKP-1 In STN

MKP-1 – role in decreasing inflammation
REM Sleep Deprivation Can Promote Prolonged Peripheral and Central Sensitization
Test the hypothesis that sensitization of the trigeminal system prior to activation of TRPA1 receptors by a pungent odor is sufficient to cause sustained hyperalgesia and allodynia.
REM Sleep Deprivation Promotes Peripheral Sensitization of Trigeminal Nociceptors

1. Sensitization of DRG Peripheral Nociceptors

2. Sensitization of Spinal Neurons and Glia

3. Sensitization of TG Peripheral Nociceptors

REM Sleep Deprivation Model

UGO Basile Durham Rat Holder
Activation of Trigeminal Nerves Via TRPA1: Headache Tree Extract

*Umbellularia californica*

**Umbellulone**

Nassini (Geppetti), Brain 2012

Pungent Odors: Trigger for migraine
REM Sleep Deprivation and Pungent Odor Increase Nocifensive Behaviors in V1 and V3

![Graph showing the effect of REM sleep deprivation and pungent odor on nocifensive behaviors in V1 and V3 areas.](image-url)
REM Sleep Deprivation Can Promote Chronic Peripheral and Central Sensitization

Sleep Deprivation → IL-1β, IL-6, NGF, PG, ATP, NO → Activation/Sensitization of primary afferents → Neuronal Cell Body → Neuropeptide Release → Trigeminal Neuron → Activation of Neurons, Astrocytes, Microglia → Pain

- Sleep loss – increased levels of proinflammatory cytokines (IL-1β, IL-6, TNF-α)
Sleep Deprivation: Consequences

Sleep problems

Causes
- psychological, physical, lifestyle and environmental

Night time

Time

Daytime

Consequences on Health and well-being

The day after
2nd disturbed night

...
Unpredictable and Chronic Stress Promote Hypervigilant State

[Diagram]

- Unpredictable
  - Severe
  - Vulnerability
- Predictable
  - Moderate
  - Resilience
Perception of Real or Presumed Physical and Social Threats Leads to Activation of HPA Axis

Front. Psychiatry, 07 August 2013
Migraine Risk Factors and Triggers

• N=1,207 patients with migraine
• 75.9% reported triggers
  – 40.4% infrequently
  – 26.7% frequently
  – 8.8% very frequently

Adapted with permission from Kelman L. Cephalalgia. 2007; 27:394–402.
Chronic Nicotine Promotes Expression of Proteins Implicated in Development of Peripheral and Central Sensitization of Trigeminal Neurons
Nicotine

- Alkaloid found in the tobacco plant (*Nicotiana tabacum*)
- Most common forms of tobacco use include chewing tobacco and cigarette smoking
- Acts on nicotinic acetylcholine receptors (nACH) localized in peripheral and central nervous system
- Strong correlation with chronic nicotine use and higher levels of pain and sensitivity
TMD/Migraine and Trigeminal Sensitization

Peripheral Sensitization
- Early: within ~20 min of the onset of pain
- Can last for up to 2 hours
- Drives central sensitization

Central Sensitization
- Later: within ~60 min of the onset of pain
- Can last up to 10 hours
- Drive the maintainance of prime-state

Primed State of Trigeminal Nociceptor
- Increased sensitivity to inflammatory stimuli
- Involves increased expression or activity of ion channels and receptors
- Sensitivity is markedly prolonged (weeks - months)

Trigeminal Ganglion Nociceptors

Nicotine

meninges

trigeminal ganglia

spinal trigeminal nucleus
Chronic Nicotine Promotes Expression of Proteins Implicated in Development of Peripheral and Central Sensitization of Trigeminal Neurons
Epigenetic Inheritance - Influences Multiple Generations

Changes in gene expression – not caused by mutations (changes in DNA sequence)

As if three generations - exposed to the same environmental conditions (diet, toxins, hormones, etc.)

Epigenetic changes - transient by nature

Epigenetic change triggered by environmental conditions may be reversed when environmental conditions change again

Smoking
Stress
Malnutrition
Hypertension
Under nutrition
Diabetes
Over nutrition
Obesity

Andrew P. Feinberg
Mechanism of Multigenerational Exposure and Transgenerational Inheritance

Figure 1 Schematic of multigenerational exposure and transgenerational inheritance.
Social Environment Affects Epigenetic Program

**EXPERIMENT #1**
1. Very attentive mothers and very inattentive mothers bred
   - Attentive mother raises biological pups
   - Inattentive mother raises biological pups
2. Researchers examine the brains of grown pups
   - Genes rarely methylated
   - Genes highly methylated
   - Hippocampus

**EXPERIMENT #2**
1. Very attentive mothers and very inattentive mothers bred
   - Pups switched at birth
   - Mothers raise foster pups
2. Researchers examine the brains of grown foster pups
   - Genes rarely methylated
   - Genes highly methylated
   - Hippocampus

**EXPERIMENT #3**
1. Inattentive mothers bred
   - Inattentive mother raises biological pups
2. Brains of “damaged” pups treated with trichostatin A, a drug that removes methyl groups
3. Epigenetic changes disappear
   - Genes rarely methylated
   - Hippocampus
   - STRESS
Early life stress, such as childhood abuse, neglect and loss, is a well established major risk factor for developing depressive disorders later in life.

<table>
<thead>
<tr>
<th>Stress exposure</th>
<th>Pathology</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal separation and stress</td>
<td>Social anxiety and recognition and stress resilience</td>
<td>Franklin et al. 2011 [43]</td>
</tr>
<tr>
<td>Traumatic paternal stress (odorant)</td>
<td>Behavioral and neural metabolic responses</td>
<td>Dias et al. 2014 [44]</td>
</tr>
<tr>
<td>Gestational restraint and forced swimming</td>
<td>Preterm birth and prenatal growth and behavior</td>
<td>Yao et al. 2014 [1]</td>
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</table>
Early-life Stress Impacts Developing Hippocampus and Primes Seizure Occurrence

DOHaD: Developmental Origins of Health and Disease

Front. Mol. Neurosci., 10 February 2014
Reducing prenatal and postnatal stress may help reduce the cost of treating adult diseases.

Ideally, intervention and prevention should be achieved before pregnancy begins.

Psychosocial interventions in early life can affect brain development and thereby benefit children at risk.

Other perinatal adversities such as perinatal infection, nutritional disorders, and toxin exposures must be cautiously avoided and treated.
Secondary Traumatic Stress Promotes Sensitization of Trigeminal Neurons

Activation/Sensitization of Primary Afferents

TMJ or Muscle Dura

Trigeminal Neuron

Neuronal Cell Body

Satellite Glia

Central Sensitization

Stress

Trigeminal Nucleus

Activation of Neurons
Astrocytes
Microglia
Model for Studying Secondary Traumatic Stress

Sender Rat
Receiver Rat
Receiver Rat

Acclimating
Swimming
Breeding
Sitting
Gestation
Swimming
Birth
Sitting
Nursing
Swimming
Weaning
Sitting
Growth
Weaning
von Frey and Sample Collection

Prenatal or Postnatal Secondary Stress Causes Prolonged Peripheral Sensitization in TG – P-ERK

Control Naïve Animals

Secondary Stressed Animals
Prenatal or Postnatal Secondary Stress Causes Prolonged Peripheral Sensitization in TG – P-p38

Control Naïve Animals

Secondary Stressed Animals
Prenatal or Postnatal Secondary Stress Causes Prolonged Central Sensitization in STN – P-ERK

Control Naïve Animals

Secondary Stressed Animals
Prenatal or Postnatal Secondary Stress Causes Prolonged Central Sensitization in STN – P-p38

Control Naïve Animals

Secondary Stressed Animals
Prenatal or Postnatal Secondary Stress Causes Prolonged Central Sensitization in STN – CGRP

Control Naïve Animals

Secondary Stressed Animals
Prenatal or Postnatal Secondary Stress Causes Prolonged Central Sensitization in STN – GFAP (Astrocytes)

Control Naïve Animals

Secondary Stressed Animals
Model for Studying Secondary Traumatic Stress

Early Life Stress Increased Basal Mechanical Sensitivity in Female Offspring

**60 g V1**

<table>
<thead>
<tr>
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<th>Average Number of Nocifensive Responses</th>
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<tbody>
<tr>
<td>Male</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>2.5</td>
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**100 g V3**

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<th>Average Number of Nocifensive Responses</th>
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<td>Male</td>
<td>0.5</td>
</tr>
<tr>
<td>Female</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Gut-Brain Axis – Integration of Multiple Systems

Stress

Brain

Gut-Brain Axis

Psychological Disorders
Abnormal Behavior
Cognition Deficit
Anxiety
Autism
Visceral Pain

Neuroendocrine Systems
Neuroimmune Systems
Autonomic Nervous System
Enteric Nervous System

Microbiota

Dysbiosis

GABA, 5-HT Precursors
SCFAs, Peptide YY
Inflammatory Cytokines

Gut

Functional GI Disorders
IBS (Ulcerative Colitis and Crohn’s Disease)
Post-Infectious IBS
Healthy Gut = Healthy Brain

Trigeminal Neurons Express SCFA Receptors
Inhibit -- Sensitization Activation
Stress Differentially Affects Microbial Populations in Cecum in Males and Females

Relative Lactobacillus Levels

- **Male:** Naïve > Stressed
- **Female:** Naïve > Stressed

Relative Bacteroides Levels

- **Male:** Naïve > Stressed
- **Female:** Naïve > Stressed
Early Life Stress Causes Major Shift in Male Microbiota

Naive Male Fecal

- actinobacteria
- firmicutes
- verrucomicrobia
- proteobacteria
- cyanobacteria
- bacteroidetes
- spirochaetes
- tenericutes
- deferribacteres

Bacteroides: Firmicutes = 0.45

Stressed Male Fecal

- actinobacteria
- firmicutes
- verrucomicrobia
- proteobacteria
- cyanobacteria
- bacteroidetes
- spirochaetes
- tenericutes
- deferribacteres

Bacteroides: Firmicutes = 0.38

Naive Male Cecal

- actinobacteria
- firmicutes
- verrucomicrobia
- proteobacteria
- cyanobacteria
- bacteroidetes
- spirochaetes
- tenericutes
- deferribacteres

Bacteroides: Firmicutes = 0.87

Stressed Male Cecal

- actinobacteria
- firmicutes
- verrucomicrobia
- proteobacteria
- cyanobacteria
- bacteroidetes
- spirochaetes
- tenericutes
- deferribacteres

Bacteroides: Firmicutes = 0.38
Early Life Stress Causes Major Shift in Female Microbiota