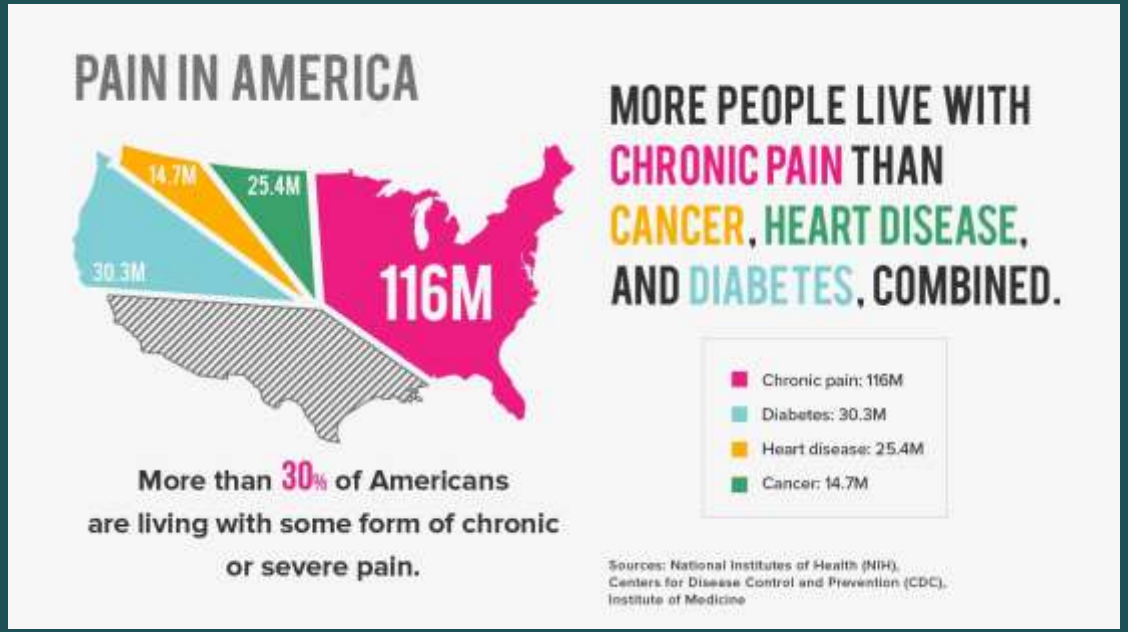
Epigenetics Role in Pain Chronification



Evolution of Migraine in Adults

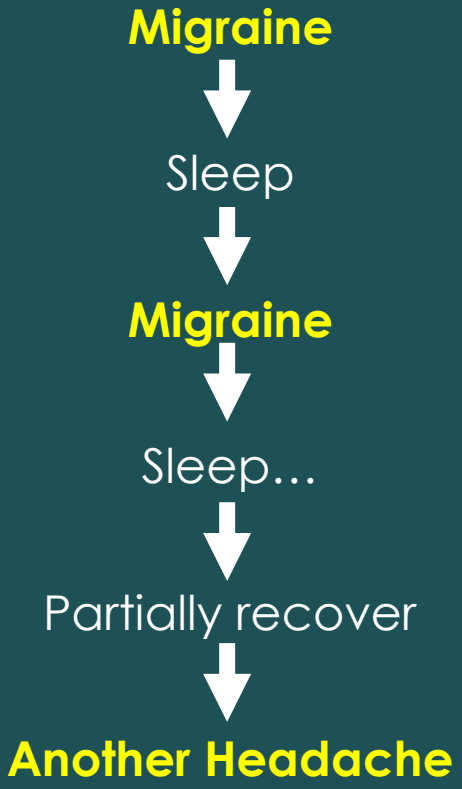
- Fortunately most people have episodic migraine
  - Most self-manage migraine
  - Gender distribution changes to approximately 6:1 (F:M) for adults in clinical trials
  - Attack frequency is higher
  - Attack disability is greater
- Chronic migraine affects 2 – 4% of the adult population

Cady RK, et al. *Headache*. 2009;49:386-394.

Episodic Migraineur

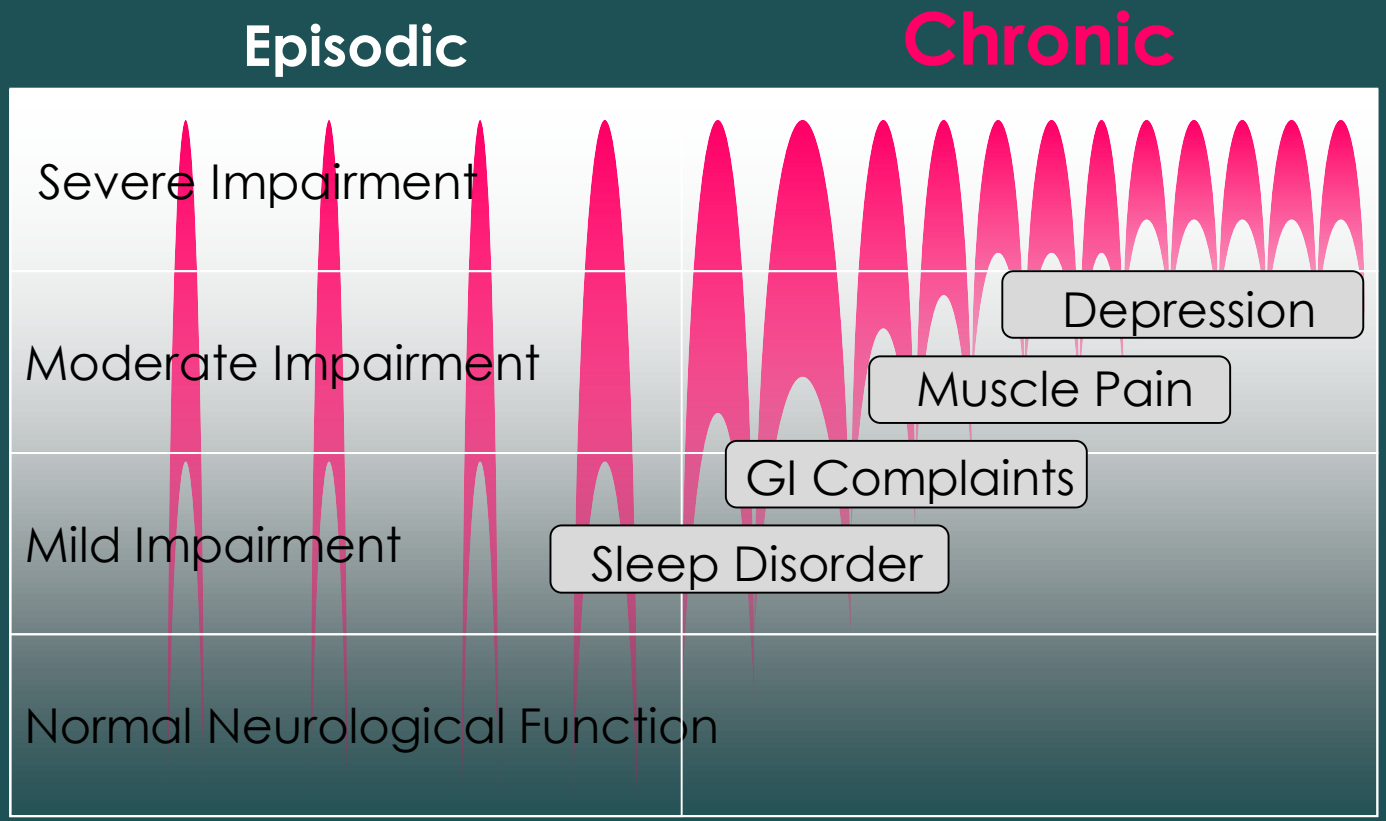
- Attacks are discrete and self limited
- Headache more localized
- Quality of pain is concise and discriminative
- Associated symptoms
  - Nausea and vomiting
  - Photo- and phonophobia
- May not become a headache patient

Chronic Migraine

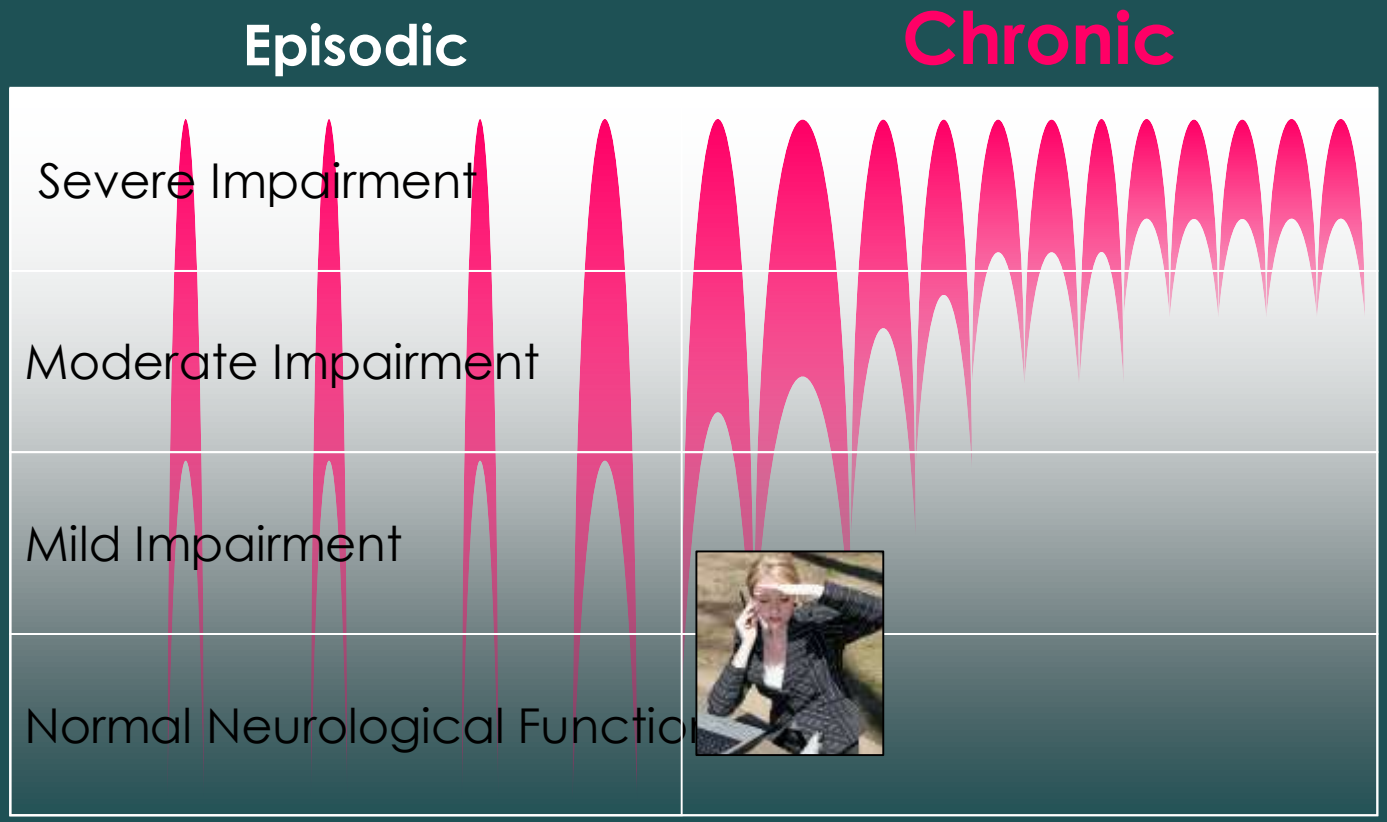


- Headaches are frequent
- Headache diffuse and variable
- Quality is mixed
- Associated symptoms
  - Nausea and vomiting
  - Photo- and phonophobia
  - Psychological
  - Myofascial
- Commonly become headache patients

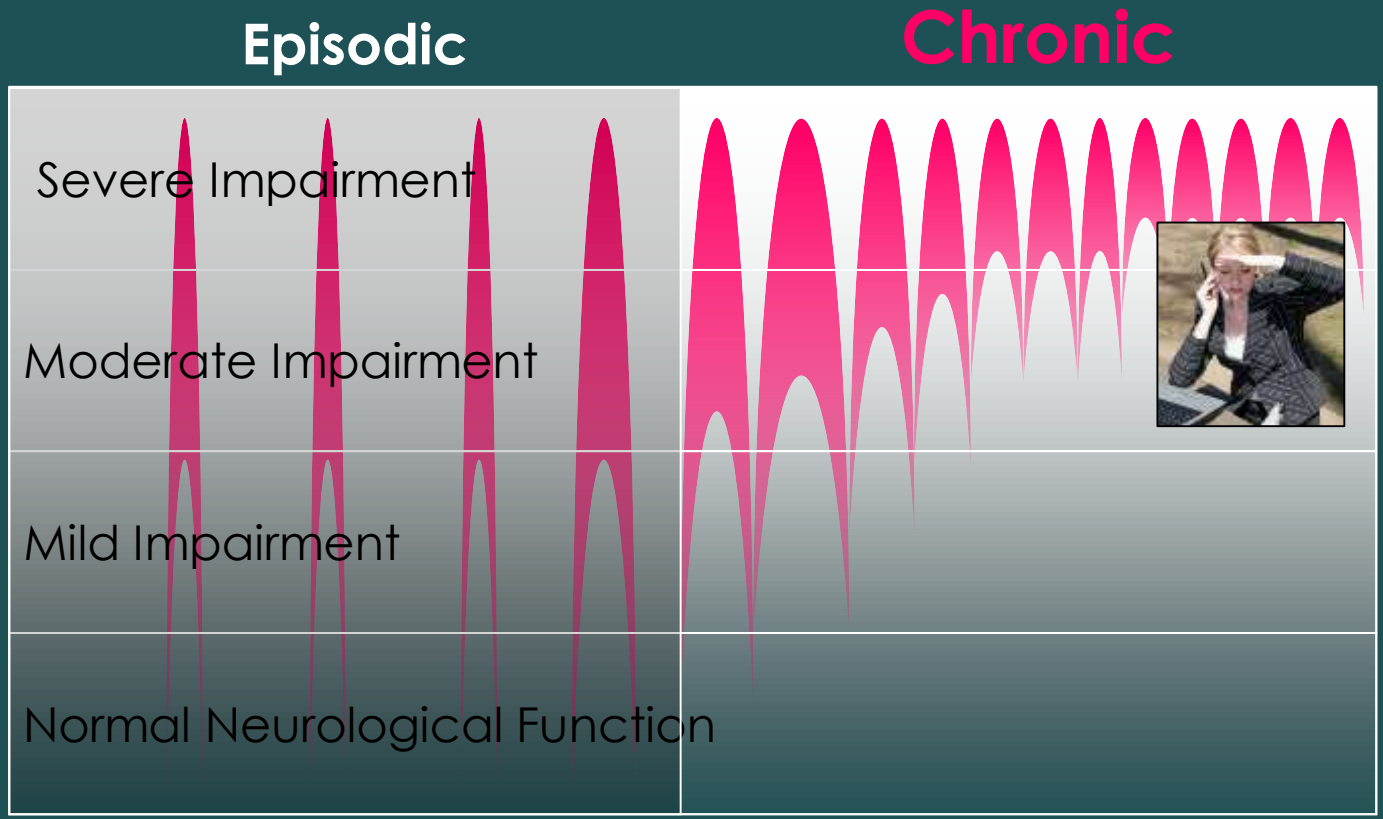
Transforming Migraine



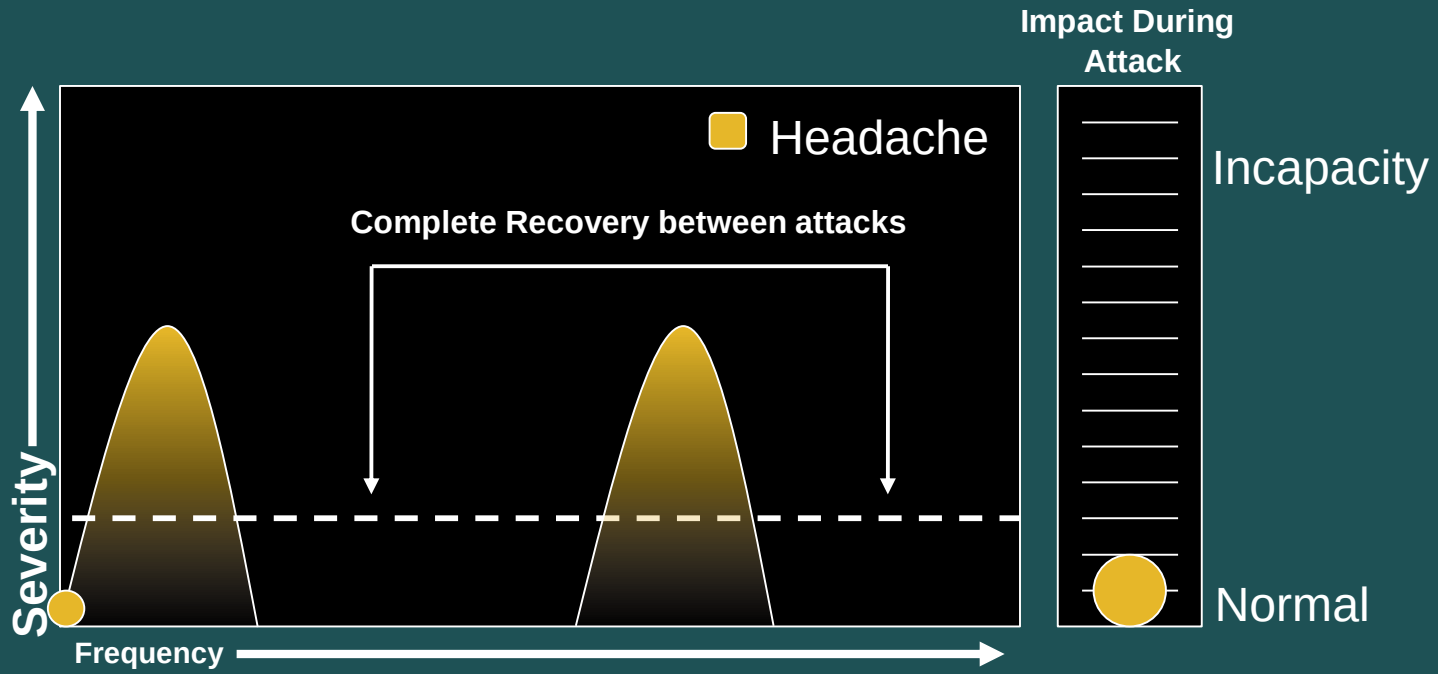
Transforming Migraine



Transforming Migraine

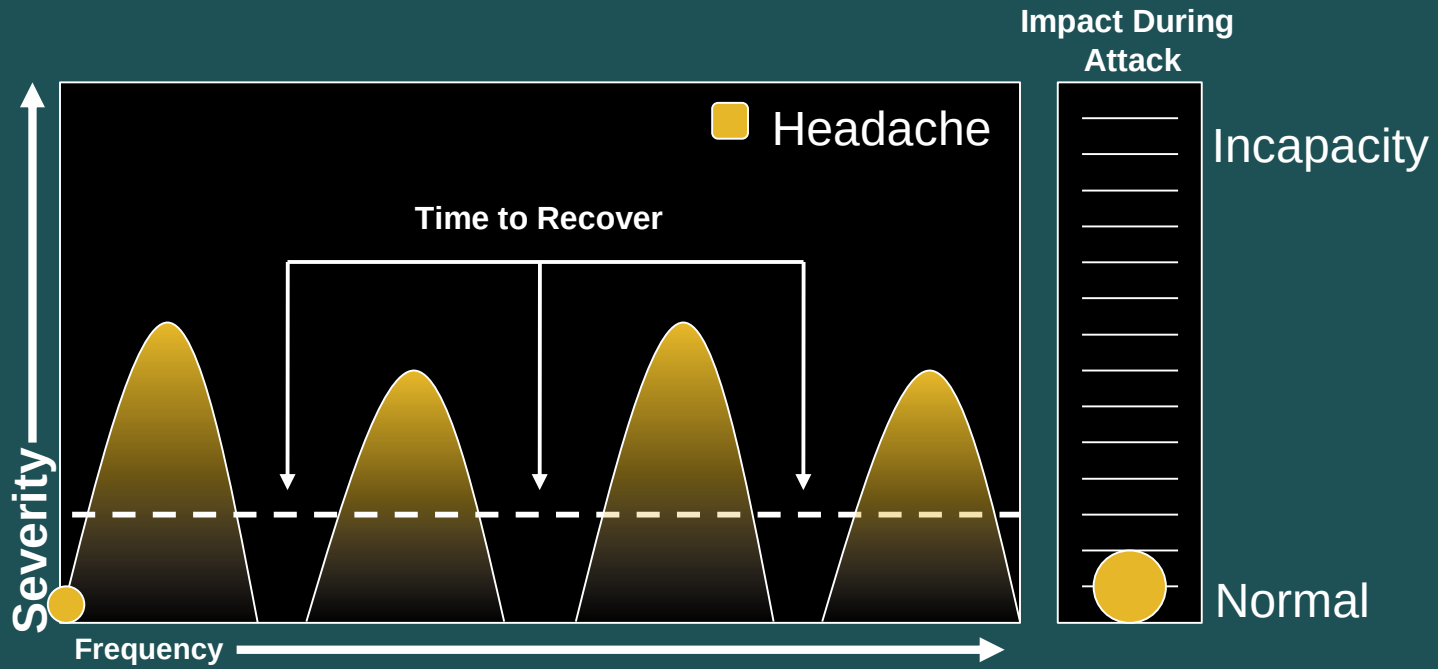


Stage 1: Infrequent Episodic Migraine



Lipton RB, et al. *Managing migraine: A healthcare professional's guide to collaborative migraine care.* Hamilton, Ontario: Baxter Publishing Inc; 2008:25.

Stage 2: Frequent Episodic Migraine



Lipton RB, et al. *Managing migraine: A healthcare professional's guide to collaborative migraine care.* Hamilton, Ontario: Baxter Publishing Inc; 2008:26.

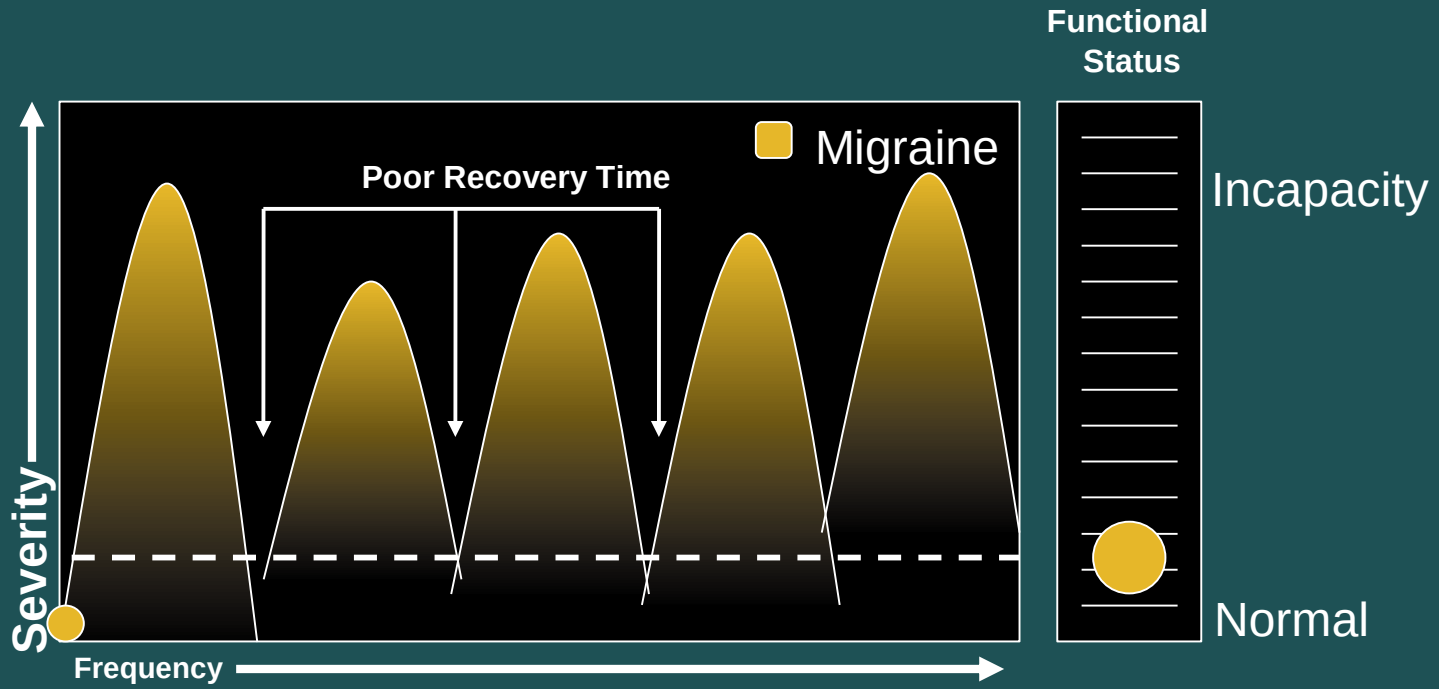
Stage 2: Migraine

- 3 or less migraine attacks per month
  or 8 HA days
- Full recovery between migraine episodes
- MIDAS generally 10 or less

MIDAS, The Migraine Disability Assessment

Lipton RB, et al. *Managing migraine: A healthcare professional's guide to collaborative migraine care.* Hamilton, Ontario: Baxter Publishing Inc; 2008:26.

Stage 3: Transforming Migraine



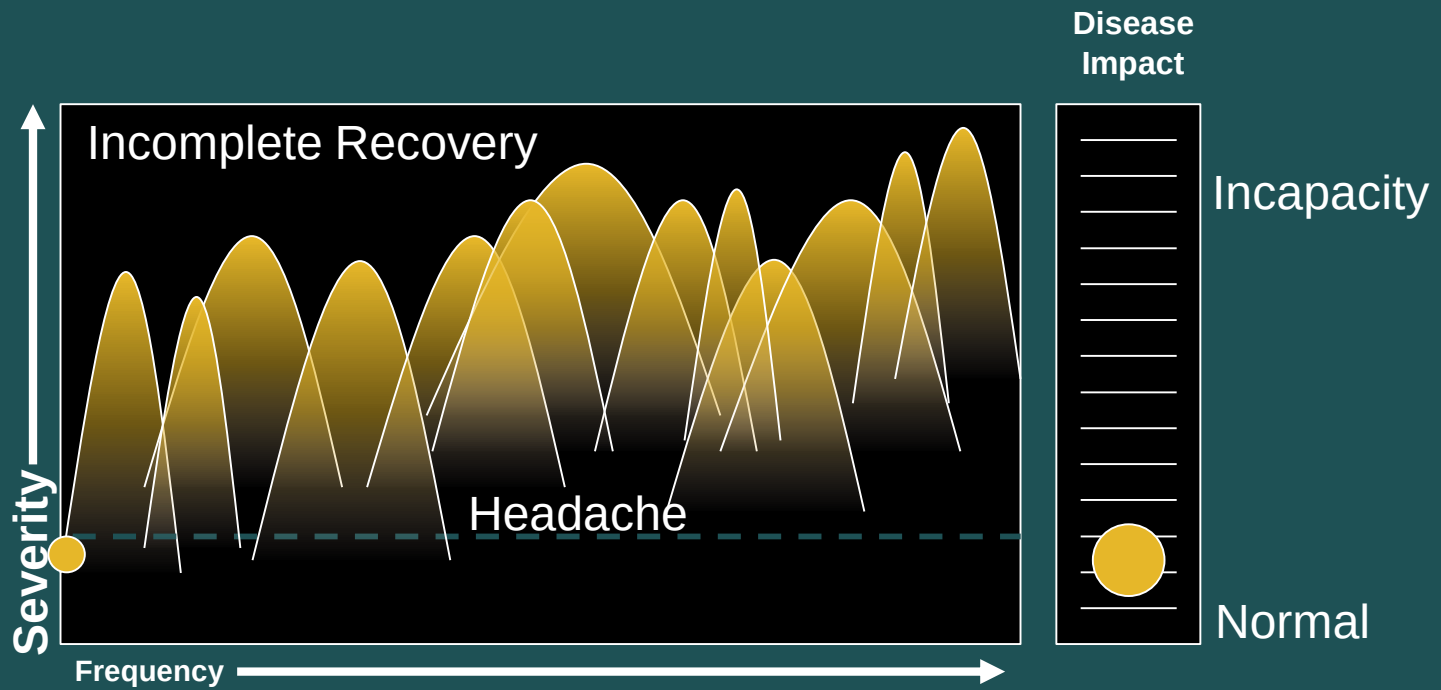
Lipton RB, et al. *Managing migraine: A healthcare professional's guide to collaborative migraine care.* Hamilton, Ontario: Baxter Publishing Inc; 2008:26.

Stage 3: Transforming Migraine

- Attacks less distinct: 8-14 days of HA per month
- Return to baseline function does not always occur between migraine attacks
- Evidence of physiological and/or psychological dysfunction often present
- MIDAS 11-20

Lipton RB, et al. *Managing migraine: A healthcare professional's guide to collaborative migraine care.* Hamilton, Ontario: Baxter Publishing Inc; 2008:26.

Stage 4: Chronic Migraine



Lipton RB, et al. *Managing migraine: A healthcare professional's guide to collaborative migraine care.* Hamilton, Ontario: Baxter Publishing Inc; 2008:27.

Stage 4: Chronic Migraine

- Greater than 15 days of HA/month for greater than 3 months (HA>4h)
- Little or no return to normal baseline function
  - Low-grade HA or feeling as if on the edge of next migraine
- Comorbidity frequent
- MIDAS 21-270

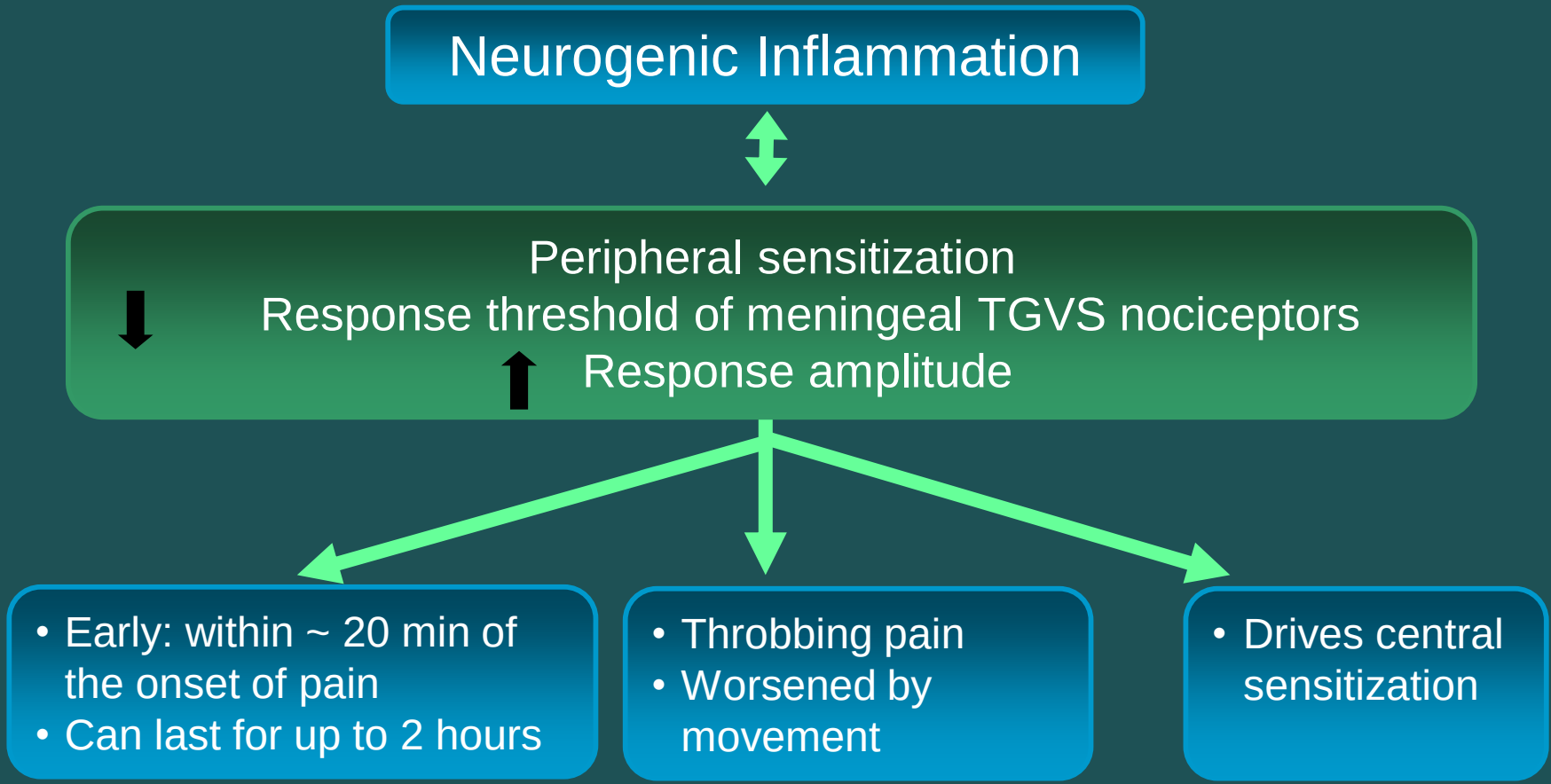
Buse DC, et al. *J Neurol Neurosurg Psychiatry*. 2010;81:428-432.
Cady R, et al. *Curr Pain Headache Rep*. 2005;9:47-52.
Blumenfeld AM, et al. *Cephalalgia.* 2011;31:301-315.

Stage 4: Chronic Migraine

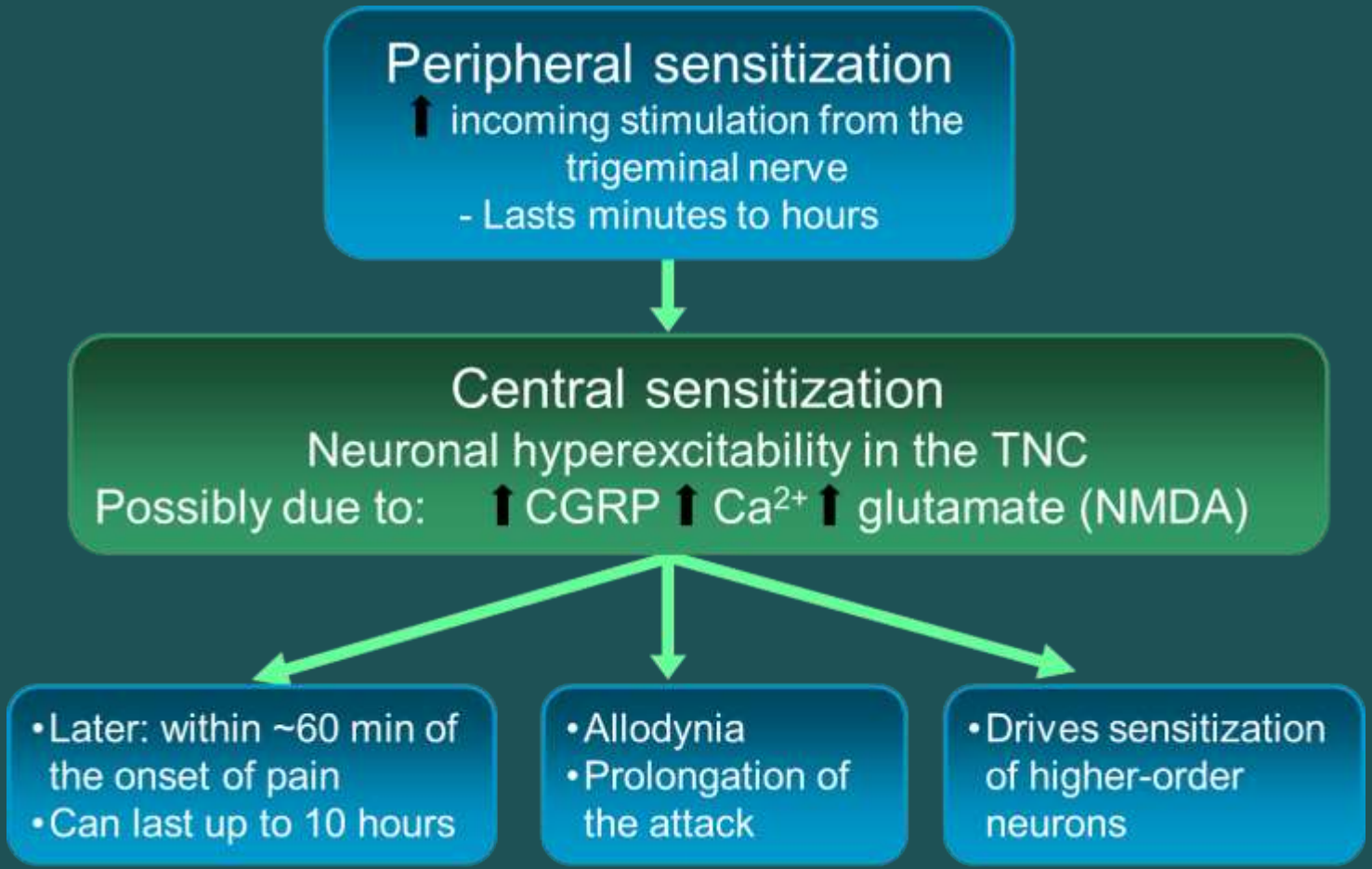
- CM is not just "more" episodic migraine
  - Greater severity of headache and associated symptoms
  - Greater impact and healthcare cost
- Delayed diagnosis and management may result in end organ damage and progression of disease
- It can be reversed!

Buse DC, et al. *J Neurol Neurosurg Psychiatry*. 2010;81:428-432.
Welch KMA, et al. *Headache*. 2001;41:629-637.

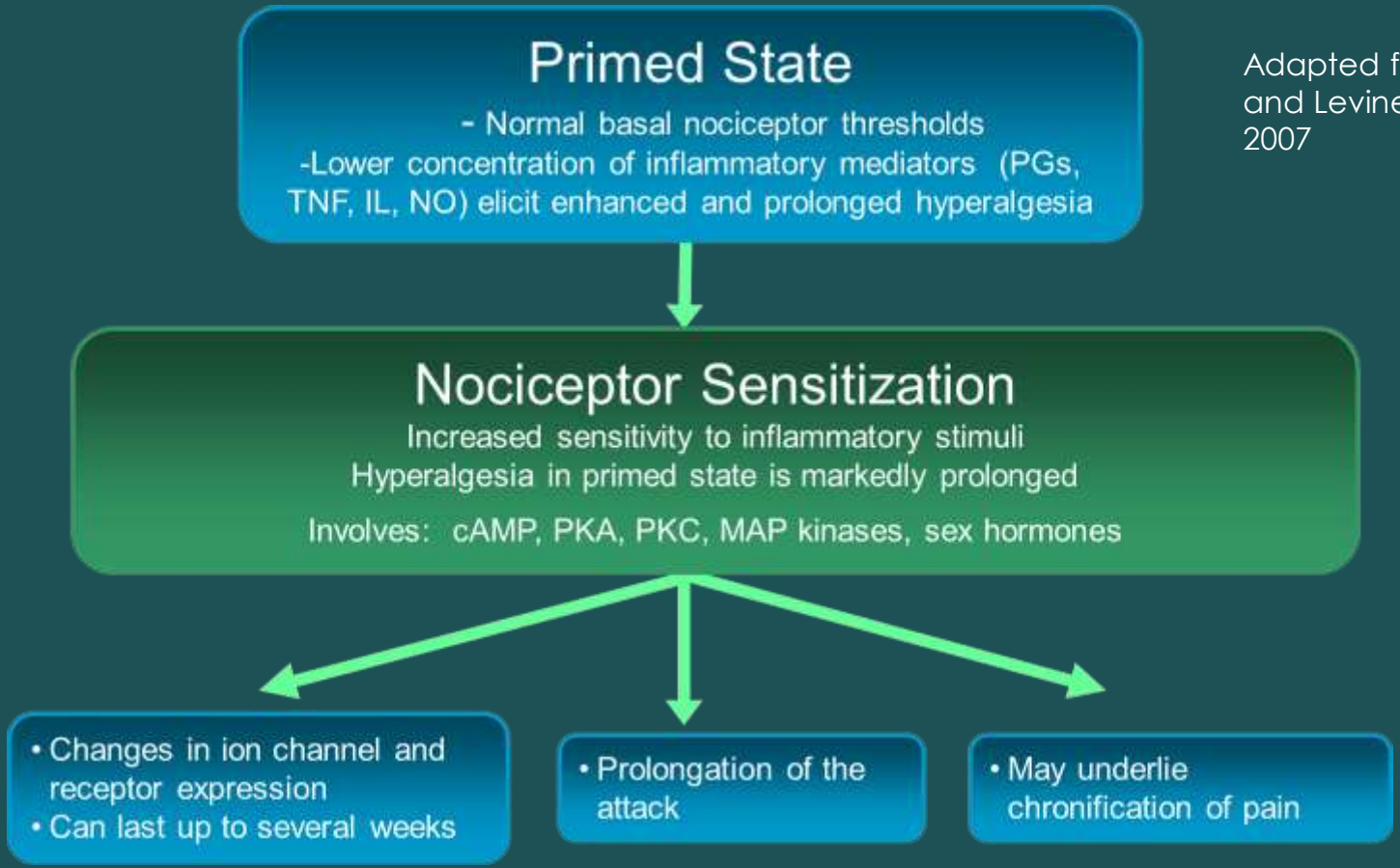
Peripheral Sensitization and TMD



Central Sensitization and TMD



Development of Primed State of Nociceptors – Chronic Sensitization

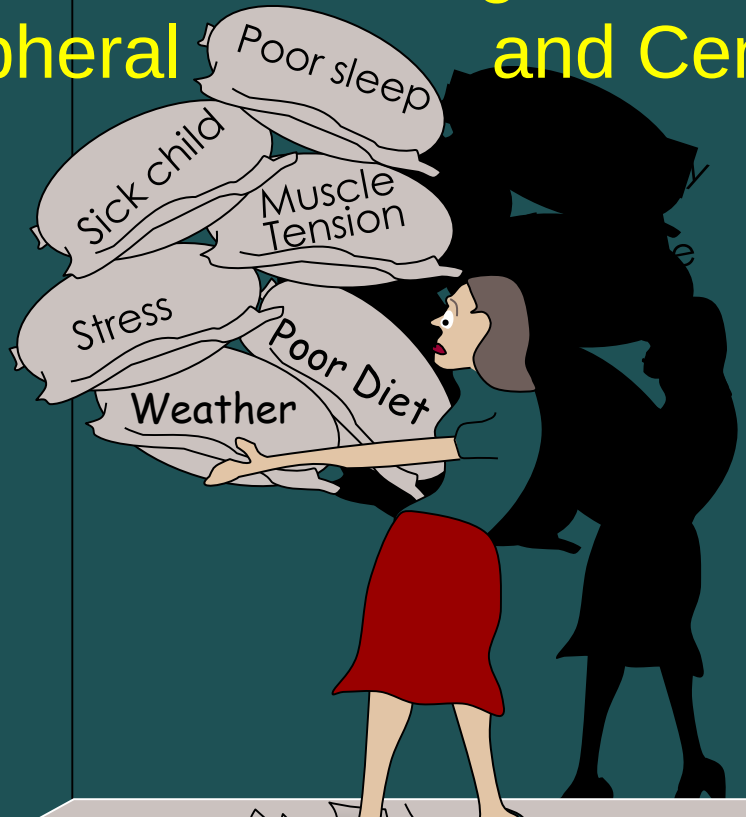


Adapted from Hucho and Levine, Neuron 55, 2007

The Perfect Storm - Increasing Number of Risk Factors Promotes Peripheral and Central Sensitization

Risk factors –

Lower activation threshold of nociceptive neurons



Triggers –

Cause activation of nociceptive neurons

Often sensory stimuli

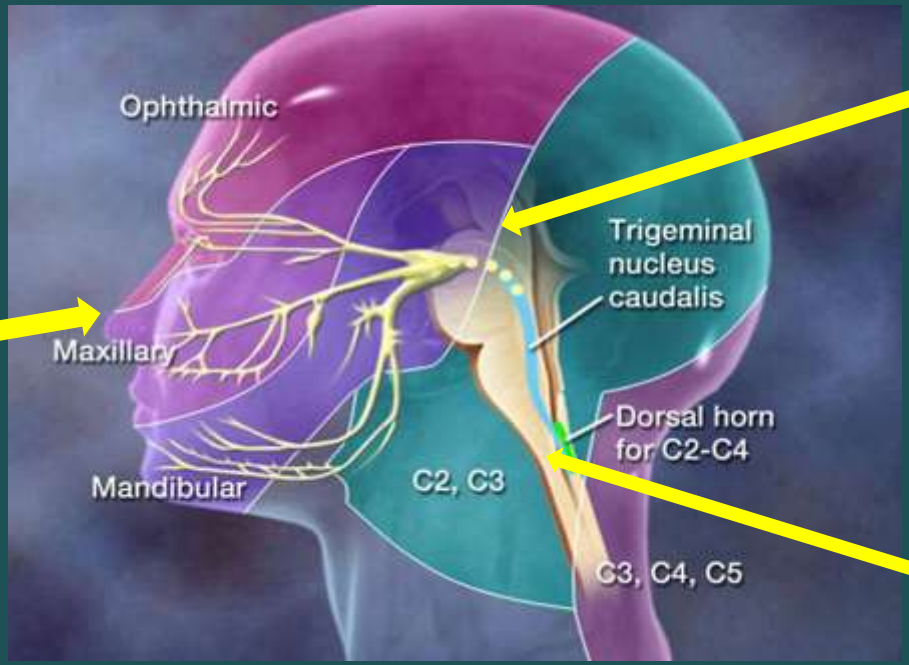
Sensitization of Trigeminal Nociceptive Neurons in Response to Prolonged Neck Muscle Pain and REM Sleep Deprivation:

Implications for Migraine and TMD Pathology (Creation of the Perfect Storm)

Risk Factors and Trigeminal Sensitization

Peripheral Sensitization

"Trigger" Headache Tree Extract



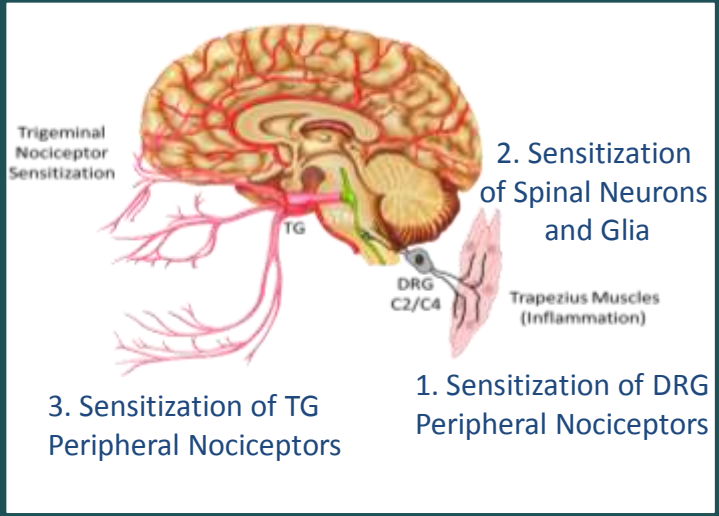
REM Sleep Deprivation

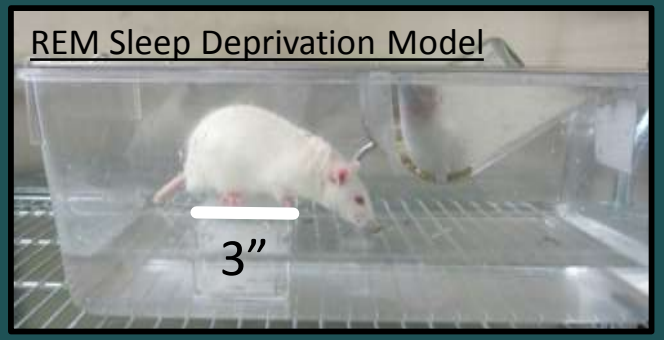
Central Sensitization

Neck Muscle Inflammation

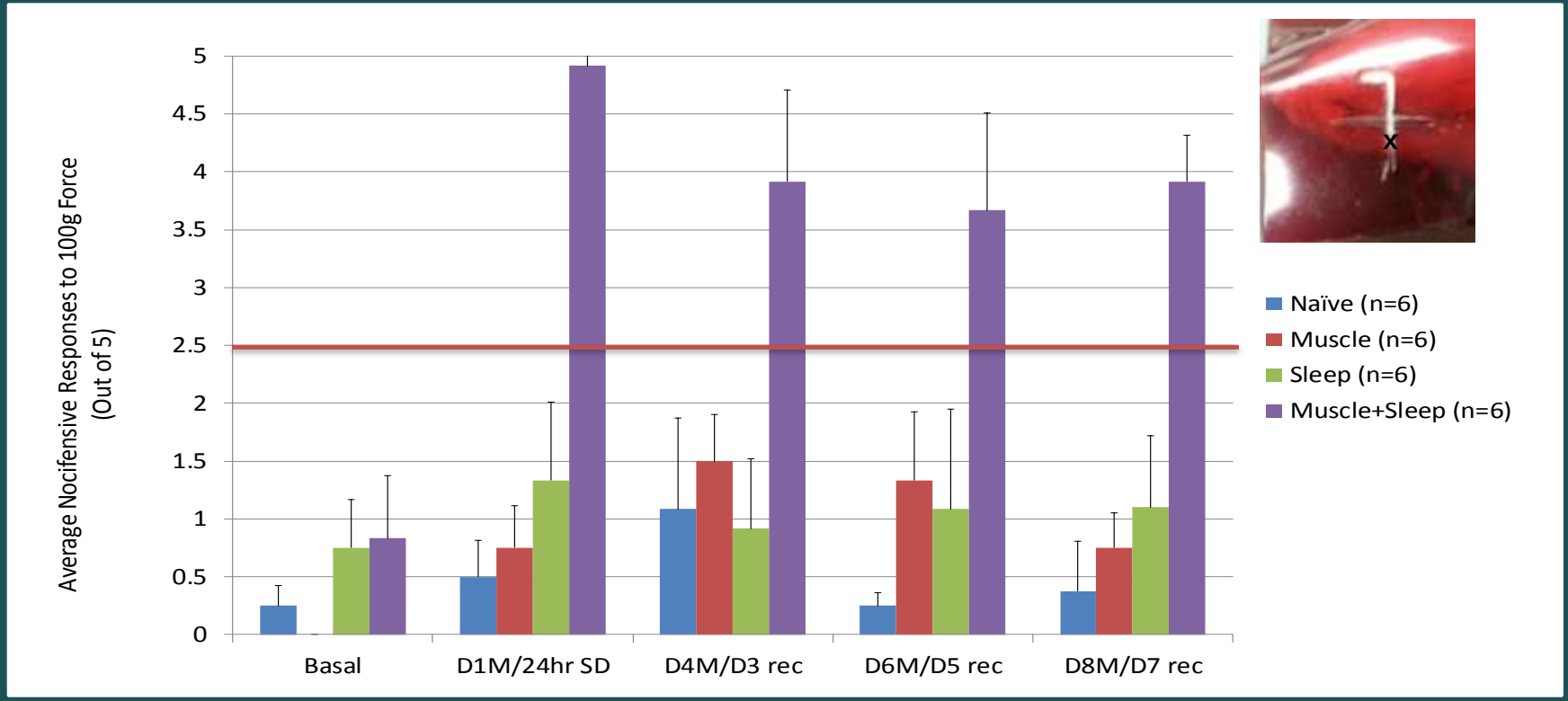
- Test the hypothesis that sensitization of the trigeminal system prior to activation of TRPA1 receptors by a pungent odor is sufficient to cause prolonged hyperalgesia and allodynia.

Neck Muscle Inflammation and REM Sleep Deprivation Promote Sensitization of Trigeminal Nociceptors



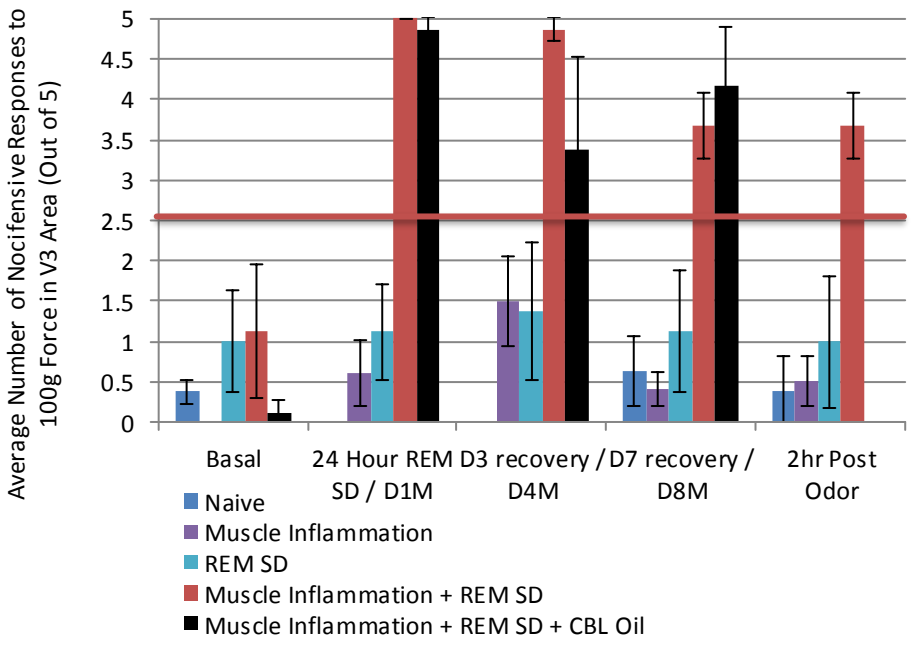


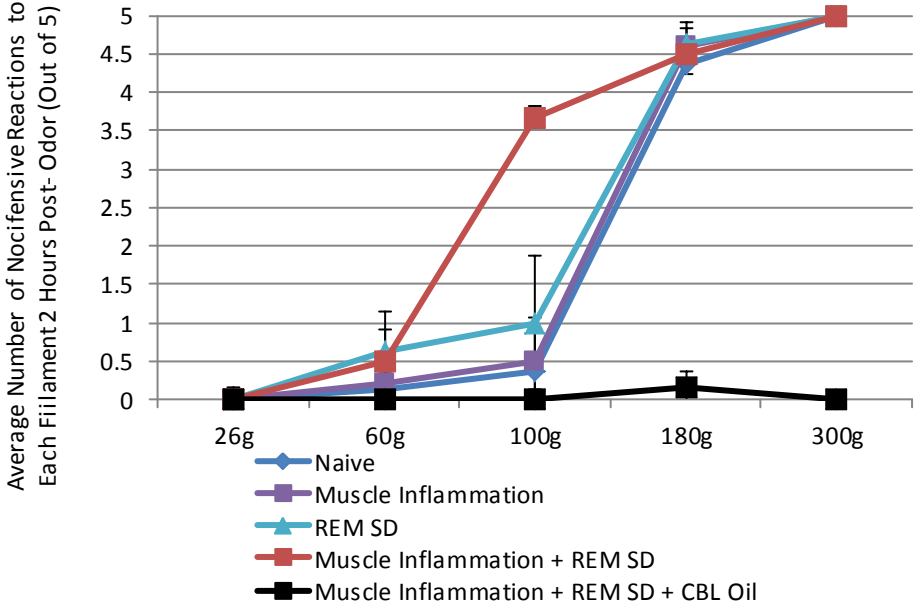
Combination of Sleep Deprivation and Inflammation of Trapezius Increase Nocifensive Responses Over the Masseter (V3)



D = day; M = muscle; SD = REM sleep deprivation; rec = recovery from sleep deprivation

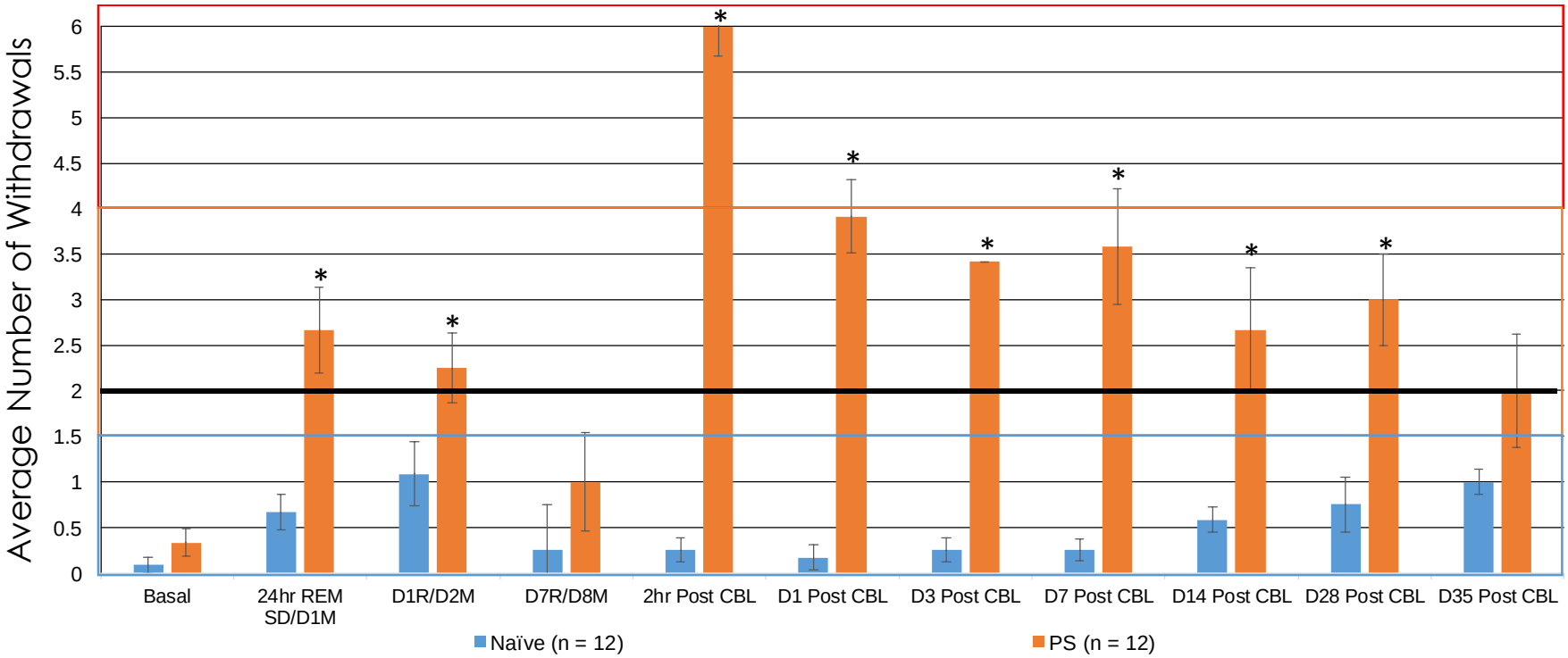
Combination of Sleep Deprivation, Inflammation of Trapezius, and Pungent Odor Results in Severe Pain Behavioral Response and Prolonged Sensitization in the Masseter Region (V3)



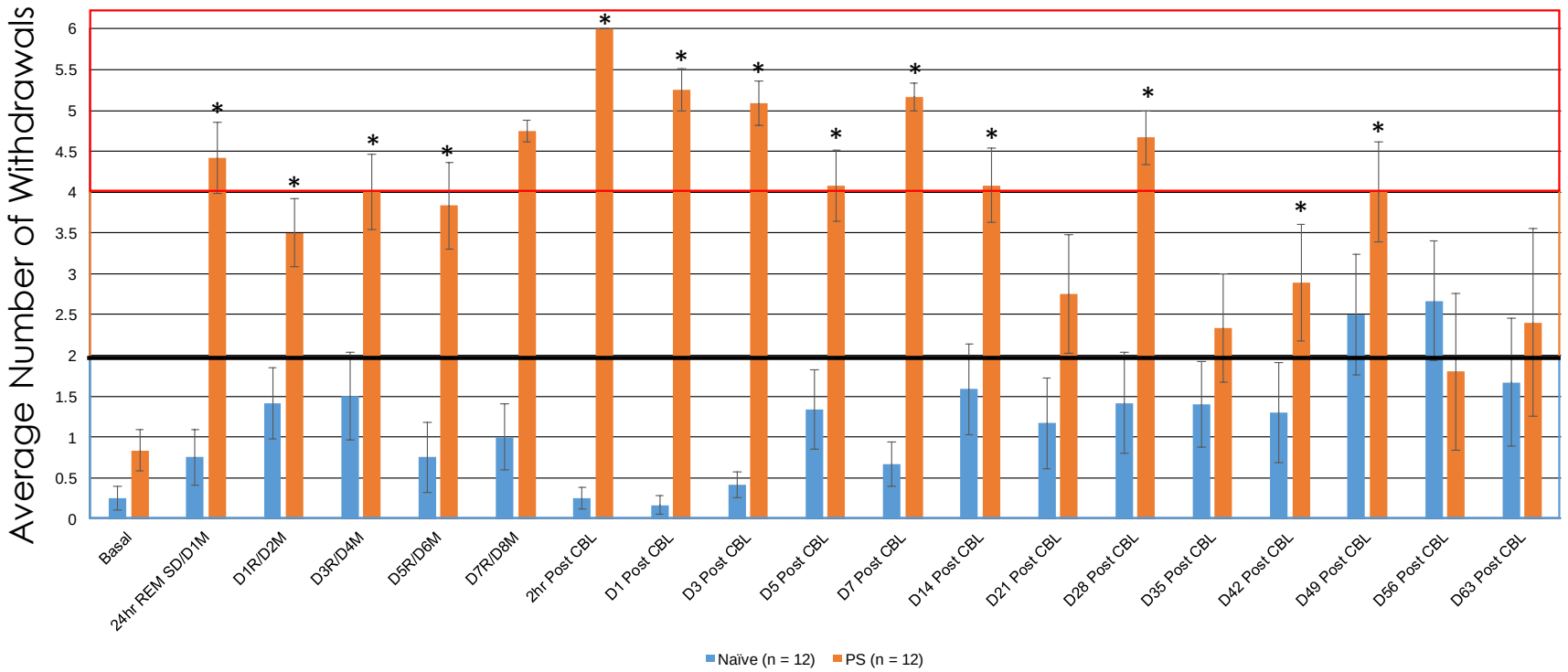


Pungent odor = extract of California Bay Leaves (“Headache Tree”) – 10 minute exposure

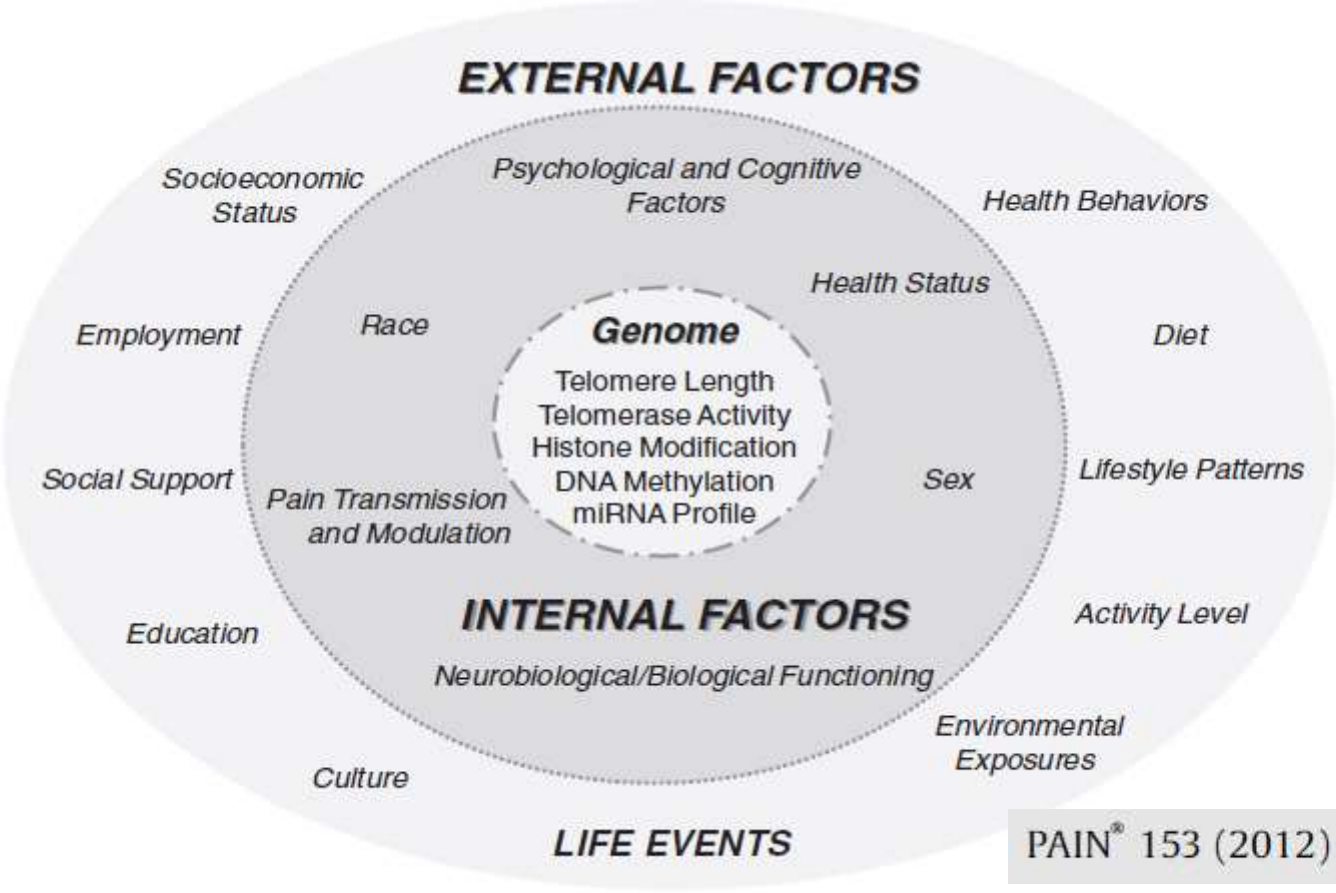
Perfect Storm – Development of Chronic Sensitized State (V1)



Perfect Storm – Development of Chronic Sensitized State (V3)

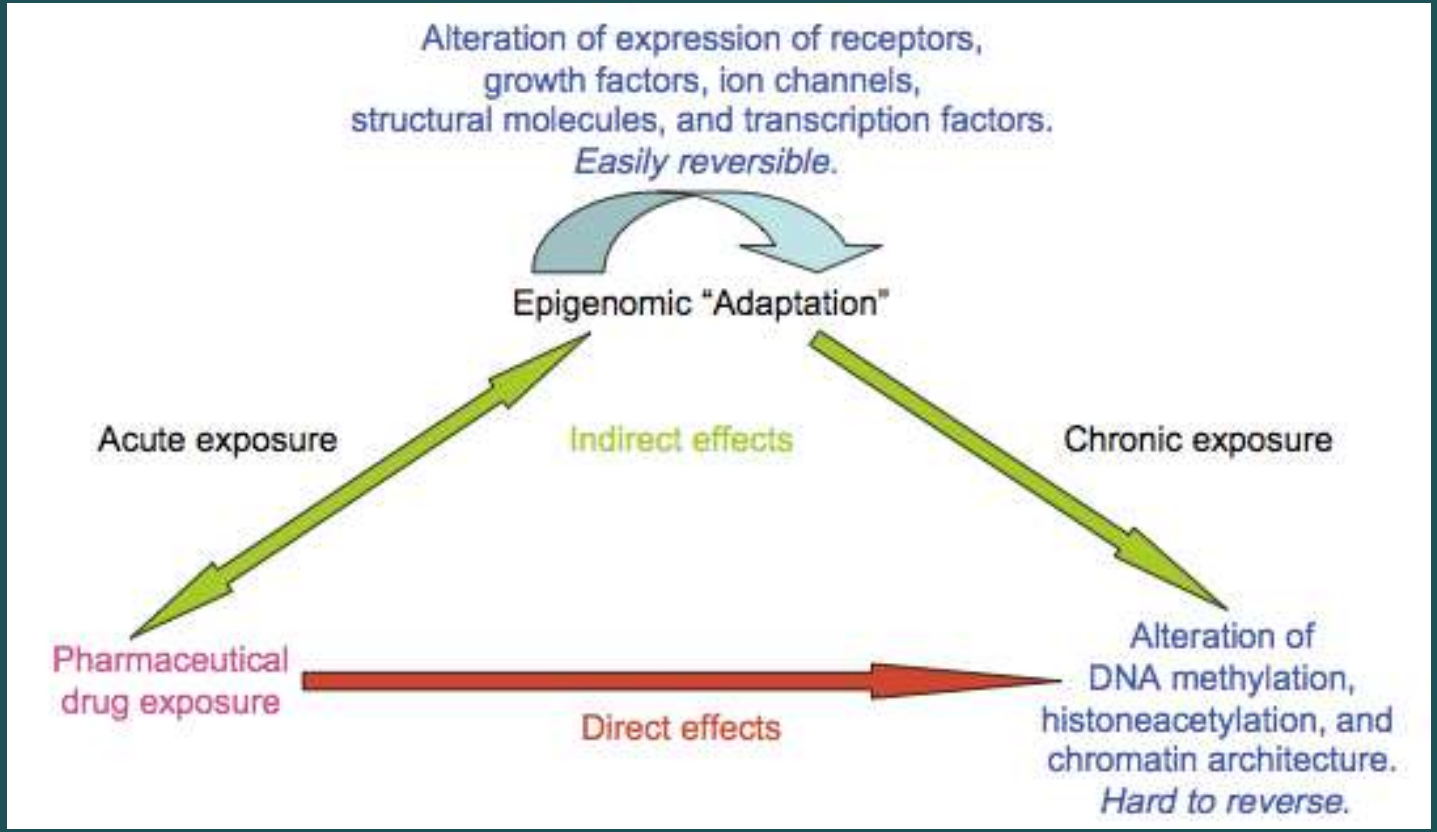


Contributors to Development of Chronic Pain

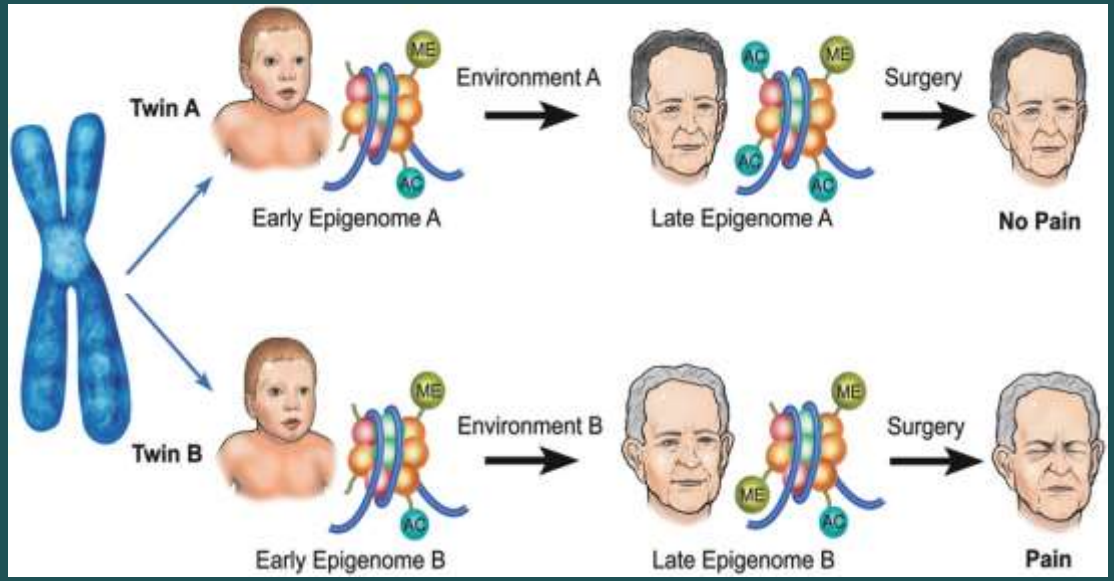


PAIN® 153 (2012) 1789–1793

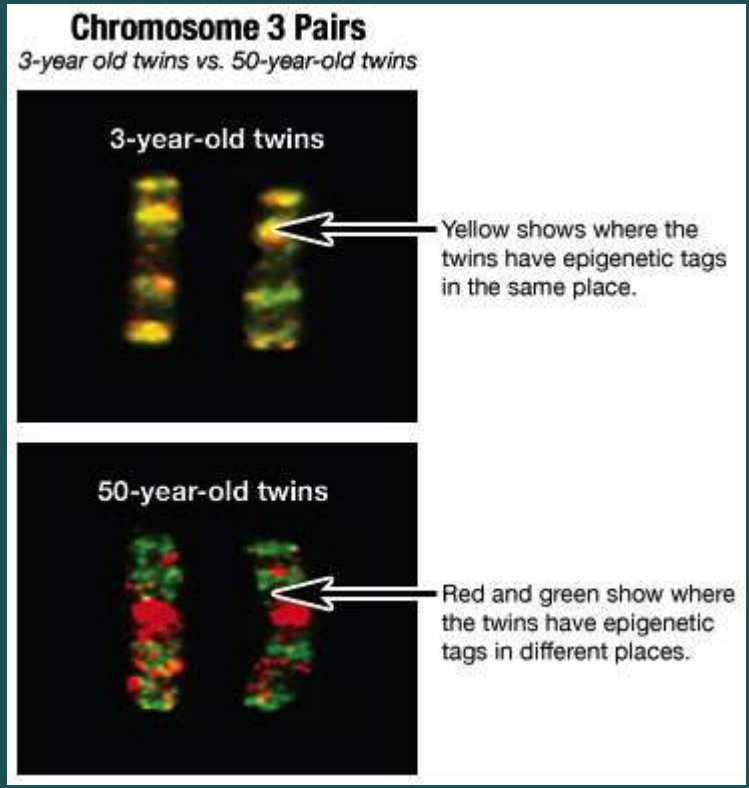
Adaptive vs Maladaptive Physiology



Epigenetics and Development of Chronic Pain - Evidence from Monozygotic Twin Studies

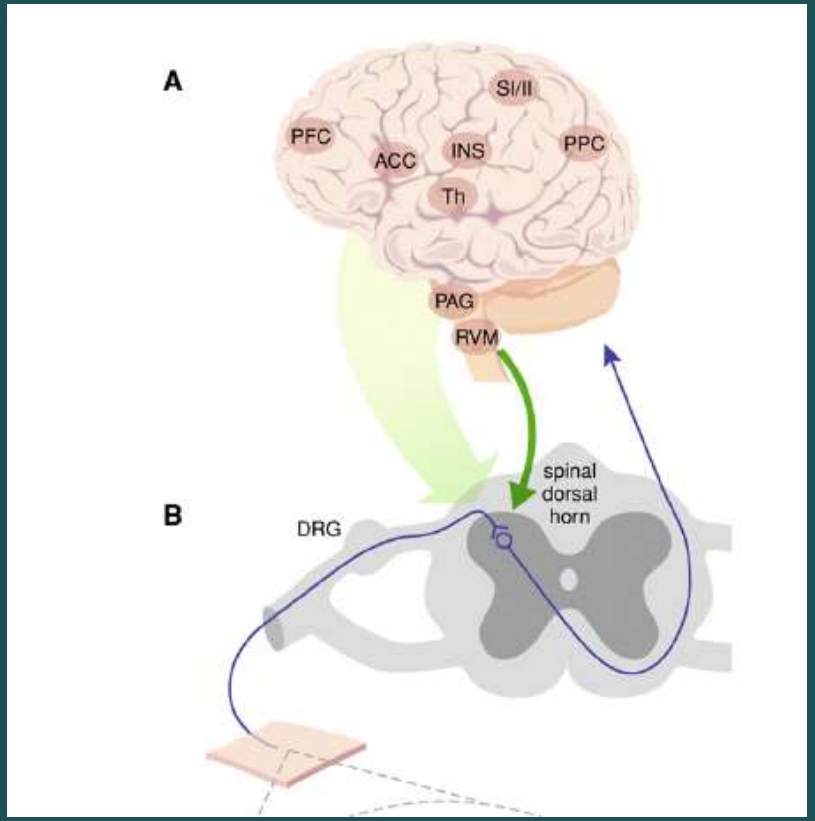


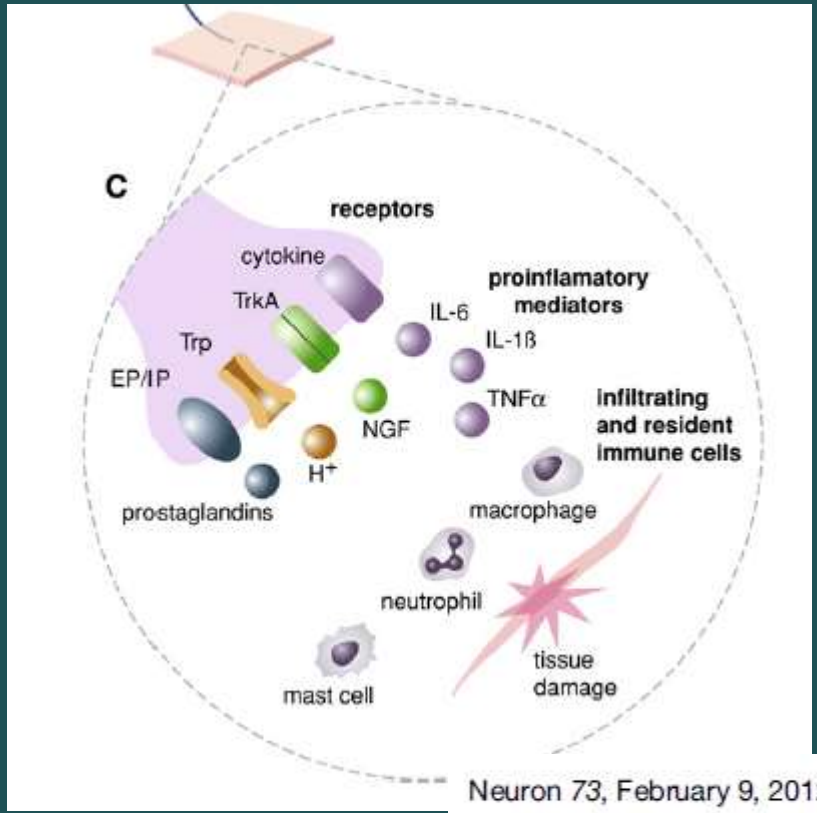
Methylation patterns similar in identical twins early in development but become altered later – evidence of environmental factors influencing gene expression and affecting health



Buchheit T, et al. *Pain Med*. 2012;13):1474-1490.

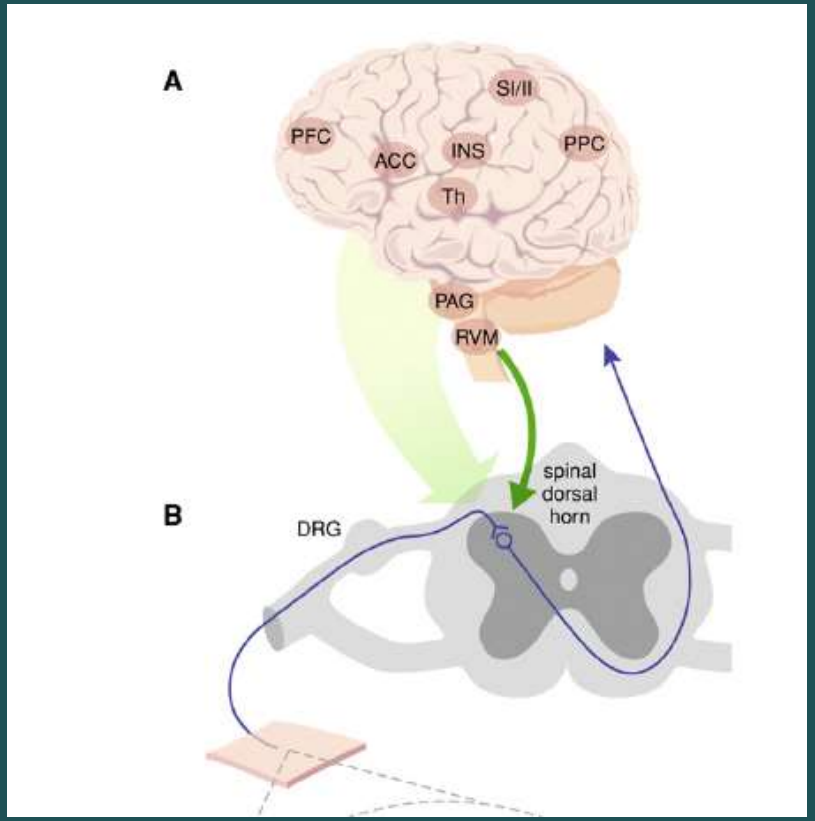
Chronic Pain: Evidence for Involvement of Epigenetics





Neuron 73, February 9, 2012 ©2012

Key Processes Thought to Underlie Chronic Pain States



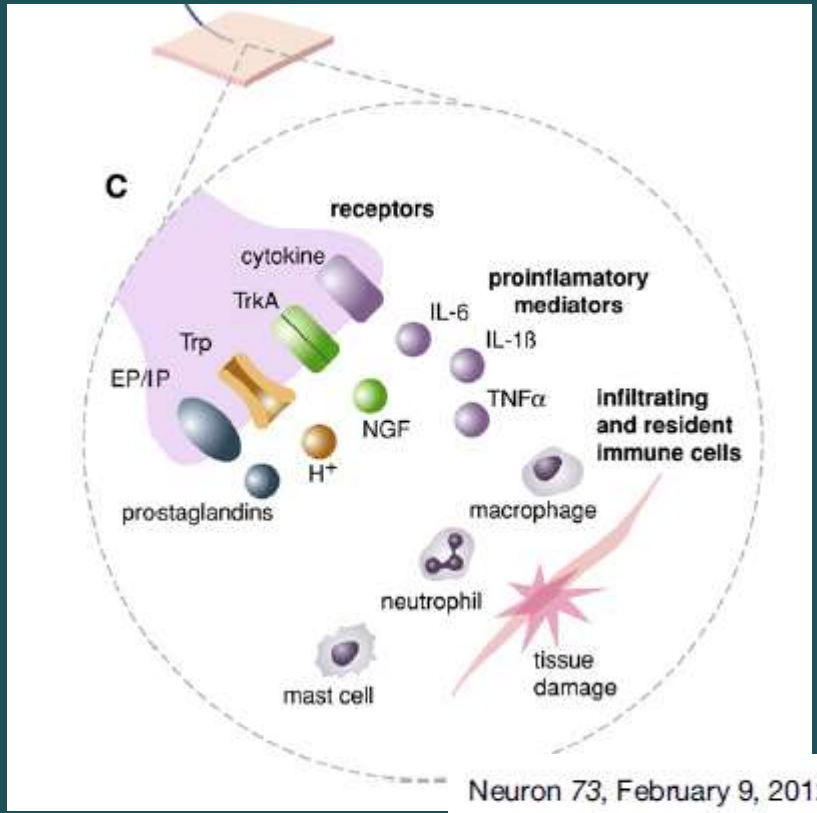
(A) Changes in brain function: a network of cortical and subcortical areas is involved in processing nociceptive signals and the sensation of pain. In chronic pain patients, many of these display profound changes in fMRI bold signal, interconnectivity, and top-down modulation of ascending spinal signals.

(B) Abnormal amplification of pain signals in DRG and spinal cord neurons: sensory neurons display hyperexcitability as a result of altered neurotrophic support and extensive changes in the expression of relevant genes, most notably ion channels and nociceptors. Second-order cells exhibit central sensitization as a result of several processes including immune and glial cell recruitment in the CNS.

Neuron 73, February 9, 2012 ©2012

Key Processes Thought to Underlie Chronic Pain States

(C) Peripheral inflammation and sensitization of nociceptors: tissue damage activates and recruits immune cells (e.g., mast cells, macrophages and neutrophils). These cells will release or stimulate the production of a variety of cytokines (e.g., IL-6, IL-1b, TNFa) and proinflammatory mediators (e.g., NGF and prostaglandins). This will activate or modulate the action of receptors on the sensory nerve terminals (e.g., the TrkA, cytokine, and prostaglandin receptors [EP/IP] are activated and Trp channels can be modulated). This process will result in sensitization of the nociceptive neuron.



Neuron 73, February 9, 2012 ©2012

Summary of Epigenetic Changes Associated With Chronic Pain States

Table 1. Summary of Epigenetic Writers, Readers, and Erasers

	Writers	*Readers*	*Erasers*
DNA methylation	DNMT1 DNMT3A DNMT3B	MeCP2 MBD1-4	not clear - only putative targets so far: - MBD2 (Bhattacharya et al., 1999) - TET enzymes leading to iterative oxydation resulting in eventual removal of methyl-cytosine (Ito et al., 2011, He et al., 2011)
Histone acetylation	***Histone acetyltransferases (HATs)*** GCN5/PCAF > H3K9/K14/K18 GNAT related (e.g.,HAT1, TFIIIC) > H4K5/K12 Myst family (e.g.,TIP60, HBO1) > H4, H3K14 CBP/p300 family > H3K14/K18 H4K5/K8 H2A, H2B TAF250 family > H3K20 SRC family (e.g., SRC1, TIF2) > H3K27	***Bromodomain proteins*** e.g., most HATs BET family (Brd2, Brd4, Bdf1) Brg-1	***Histone deacetylases (HDACs)*** class I (HDAC1, HDAC2, HDAC3, HDAC8) class IIa (HDAC4, HDAC5, HDAC7, HDAC9) class IIb (HDAC6, HDAC10) sirtuins (SIRT1 - SIRT7) class IV (HDC11)

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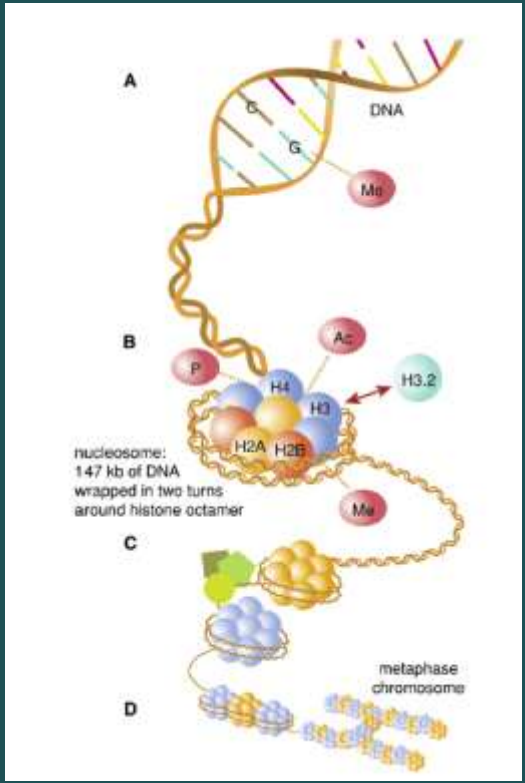
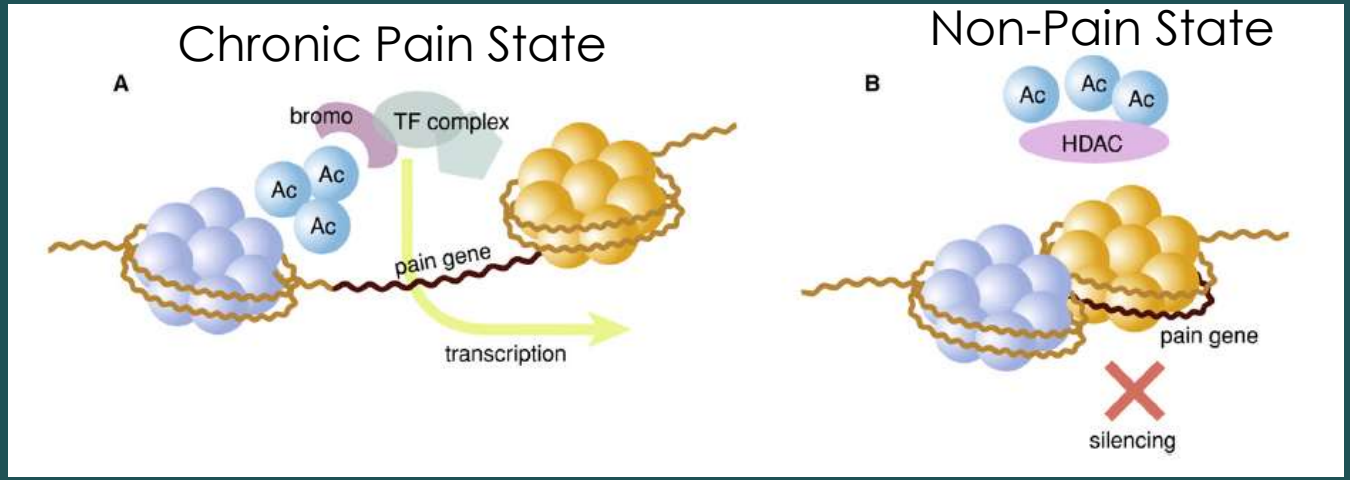
Summary of Epigenetic Changes Associated With Chronic Pain States

Histone methylation	***Lysine methyltransferases (KMTs)***		***Royal family***	***Lysine demethylases (KDMs)***
	KMT1A - KMT1F (e.g., G9a, GLP)	> H3K9	- chromo-domain proteins, e.g., HP-1 like, polycomb like, CHD like	LSD1/ KDM1
	MLL family (e.g., MLL1, hSET1A)	> H3K4		JHDM/Jumonji (e.g., JHDM1A/B, JHDM2A/B, JHDM3A-D, JARID1A-D, UTX)
	KMT3A - KMT3C (e.g., NSD1)	> H3K36	- tudor-domain proteins, e.g., SMN	
	DOT1	> H3K79		
	KMT5A, KMT5B (e.g., SUV420H1)	> H3K20	***PHD proteins***	
	KMT6/ EZH2	> H3K27	e.g., CBD, ING2, DNMT3L, PHF6	
	KMT7/ SET7&9	> H3K4		
	KMT8/ RIZ1	> H3K9		
Histone phosphorylation	***Serine/Threonine Kinases*** e.g.,		***14-3-3 proteins*** seven isoforms: theta, gamma, zeta, eta, epsilon, beta, mu	***Protein Phosphatases*** e.g., Serine/ Threonine protein phosphatases (PPP2CA, PPP2CB, PPP1CC) Protein phosphatase 1D Eye-absent homologues (EYA1-3)
	MST, AMPK	> H2B		
	Haspin, VRK, Aurora B	> H3		
	PKCα, PKCβ, MSK1/2, JNK	> H3		

Large families of proteins have been identified that add the various epigenetic marks (writers), remove them (erasers), and bind them to exert down-stream effects (readers). This table does not provide an exhaustive list, and many issues are still under debate such as the existence of active DNA demethylation (Bhattacharya et al., 1999; Ito et al., 2011; He et al., 2011). In the case of histone writer molecules, there tends to be quite a clear preference for particular lysine residues, the identity of which is also indicated here (e.g., H3K9 indicates preferential action at lysine residue 9 of histone 3). Current evidence suggests that the same preference does not exist for histone erasers. It is important to bear in mind that many of these molecules do not exclusively act on histones or even in the nucleus, but that they are also capable of modifying cytoplasmic proteins (e.g. tubulin; for review, see Sadoul et al., 2011). Hence drugs targeting their function, such as HDAC inhibitors, can also affect nonepigenetic processes.

One Possible Mechanism by Which Epigenetic Processes Promote Chronic Pain





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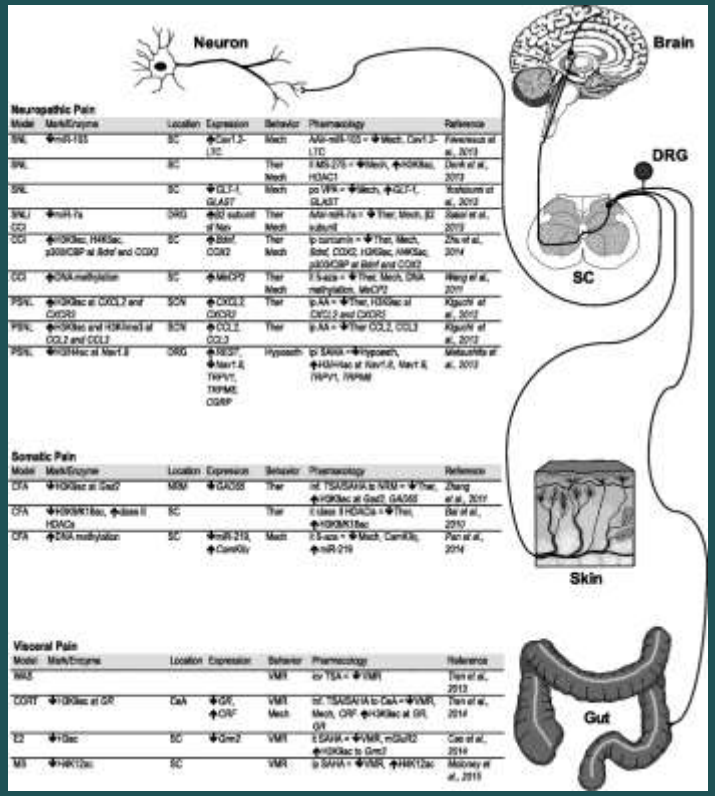
Summary of Epigenetic Changes During Various Chronic Pain Conditions and Effects of Pharmacological Treatment

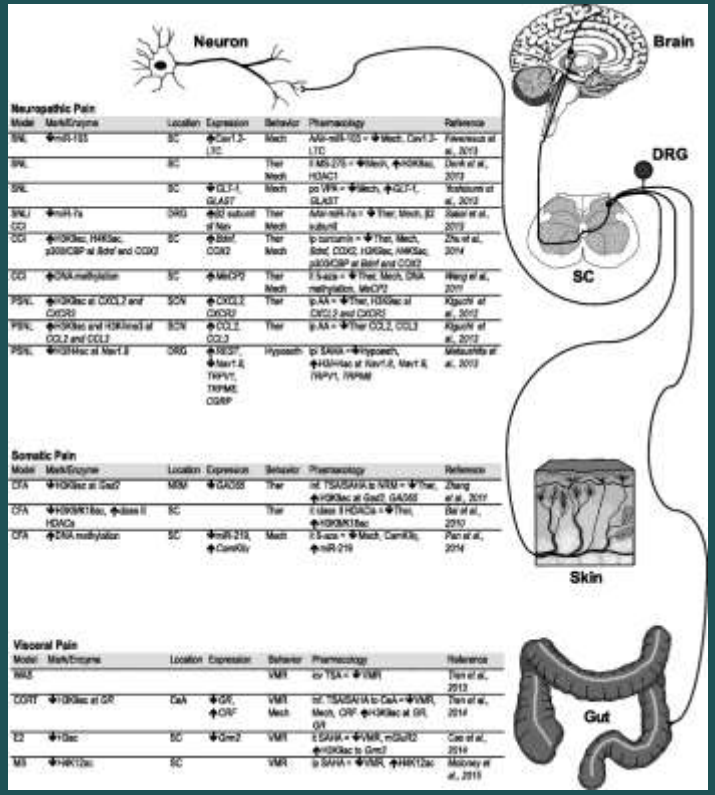


Neuropathic Pain

Model	Mark/Enzyme	Location	Expression	Behavior	Pharmacology	Reference
SNL	↓miR-103	SC	↑Cav1.2-LTC	Mech	AAV-miR-103 = ↓Mech, Cav1.2-LTC	Favereaux et al., 2013
SNL		SC		Ther Mech	i.t MS-275 = ↓Mech, ↑H3K9ac, HDAC1	Denk et al., 2013
SNL		SC	↓GLT-1, GLAST	Mech	po VPA = ↓Mech, ↑GLT-1, GLAST	Yoshizumi et al., 2013
SNL/ CCI	↓miR-7a	DRG	↑β2 subunit of Nav	Ther Mech	AAV-miR-7a = ↓Ther, Mech, β2 subunit	Sakai et al., 2013
CCI	↑H3K9ac, H4K5ac, p300/CBP at *Bdnf* and *COX2*	SC	↑*Bdnf*, *COX2*	Ther Mech	ip curcumin = ↓Ther, Mech, *Bdnf*, *COX2*, *H3K9ac*, *H4K5ac*, *p300/CBP at Bdnf and COX2*	Zhu et al., 2014
CCI	↑DNA methylation	SC	↑*MeCP2*	Ther Mech	i.t 5-aza = ↓Ther, Mech, DNA methylation, *MeCP2*	Wang et al., 2011
PSNL	↑H3K9ac at *CXCL2 and CXCR2*	SCN	↑*CXCL2*, *CXCR2*	Ther	ip AA = ↓Ther, H3K9ac at *CXCL2 and CXCR2*	Kiguchi et al., 2012
PSNL	↑H3K9ac and H3K4me3 at *CCL2 and CCL3*	SCN	↑*CCL2*, *CCL3*	Ther	ip AA = ↓Ther CCL2, CCL3	Kiguchi et al., 2013
PSNL	↓H3/H4ac at *Nav1.8*	DRG	↑*REST*, ↓*Nav1.8*, *TRPV1*, *TRPM8*, *CGRP*	Hypoesth	ipi SAHA =↓Hypoesth, ↑H3/H4ac at *Nav1.8*, *Nav1.8*, *TRPV1*, *TRPM8*	Matsushita et al., 2013

Casey O. Ligon et al. J Pharmacol Exp Ther 2016;357:84-93

Summary of Epigenetic Changes During Various Chronic Pain Conditions and Effects of Pharmacological Treatment

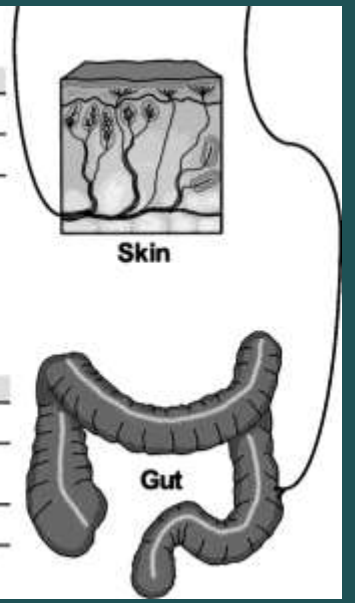


Somatic Pain

Model	Mark/Enzyme	Location	Expression	Behavior	Pharmacology	Reference
CFA	↓H3K9ac at *Gad2*	NRM	↓*GAD65*	Ther	Inf. TSA/SAHA to NRM = ↓Ther, ↑H3K9ac at *Gad2*, *GAD65*	*Zhang et al., 2011*
CFA	↓H3K9/K18ac, ↑class II HDACs	SC		Ther	it class II HDACIs = ↓Ther, ↑H3K9/K18ac	*Bai et al., 2010*
CFA	↑DNA methylation	SC	↓miR-219, ↑CamKIIγ	Mech	it 5-aza = ↓Mech, CamKIIγ, ↑miR-219	*Pan et al., 2014*

Visceral Pain

Model	Mark/Enzyme	Location	Expression	Behavior	Pharmacology	Reference
WAS				VMR	icv TSA = ↓VMR	*Tran et al., 2013*
CORT	↓H3K9ac at *GR*	CeA	↓*GR*, ↑*CRF*	VMR Mech	Inf. TSA/SAHA to CeA = ↓VMR, Mech, *CRF* ↑H3K9ac at *GR*, *GR*	*Tran et al., 2014*
E2	↓H3ac	SC	↓*Grm2*	VMR	it SAHA = ↓VMR, mGluR2 ↑H3K9ac to *Grm2*	*Cao et al., 2014*
MS	↓H4K12ac	SC		VMR	ip SAHA = ↓VMR, ↑H4K12ac	*Moloney et al., 2015*



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Summary of HDAC and DNMT Inhibitors

HDAC inhibitors	Hydroxamates	SAHA (vorinostat)		Pan inhibitor
		PXD101 (belinostat)	(*E*)-*N*-hydroxy-3-[3-(phenylsulfamoyl)phenyl]prop-2-enamide	Pan inhibitor
		LBH589 (panobinostat)	(*E*)-*N*-hydroxy-3-[4-[[2-(2-methyl-1*H*-indol-3-yl)ethylamino]methyl]phenyl]prop-2-enamide	Classes I and II
		ITF2357 (givinostat)	[6-(diethylaminomethyl)naphthalen-2-yl]methyl-*N*-[4-(hydroxycarbamoyl)phenyl]carbamate;hydrate;hydrochloride	Pan inhibitor
		4SC-201 (resminostat)	(*E*)-3-[1-[4-[(dimethylamino)methyl]phenyl]sulfonylpyrrol-3-yl]-*N*-hydroxyprop-2-enamide	Pan inhibitor
		PCI-24781 (abexinostat)	3-[(dimethylamino)methyl]-*N*-[2-[4-(hydroxycarbamoyl)phenoxy]ethyl]-1-benzofuran-2-carboxamide	Classes I and II

Summary of HDAC and DNMT Inhibitors

Cyclic peptides	Depsipeptide/FK228	(1*S*,4*S*,7*Z*,10*S*,16*E*,21*R*)-7-ethylidene-4,21-di(propan-2-yl)-2-oxa-12,13-dithia-5,8,20,23-tetrazabicyclo[8.7.6]tricos-16-ene-3,6,9,19,22-pentone	Class I	
	Benzamides	MS-275 (entinostat)	*N*-[[4-[[(2-aminophenyl)amino]carbonyl]phenyl]methyl]-3-pyridinylmethyl ester, carbamic acid	Class I
		MGCD0103 (mocetinostat)	*N*-(2-aminophenyl)-4-[[(4-pyridin-3-ylpyrimidin-2-yl)amino]methyl]benzamide	Class I
		M344	4-(dimethylamino)-*N*-[7-(hydroxyamino)-7-oxoheptyl]benzamide	Class I
	Aliphatic fatty acids	Valproate		Classes I and IIa
		Butyrate		Classes I and IIa
	Unknown mechanism	CI-994	4-acetamido-*N*-(2-aminophenyl)benzamide	HDAC1, HDAC2
		BML-210	*N*′-(2-aminophenyl)-*N*-phenyloctanediamide	HDAC 1–5 and 7
		NVP-LAQ824	(*E*)-*N*-hydroxy-3-[4-[[2-hydroxyethyl-[2-(1*H*-indol-3-yl)ethyl]amino]methyl]phenyl]prop-2-enamide	Unknown

Summary of HDAC and DNMT Inhibitors

DNMT inhibitors		5-Azacitidine		Pan inhibitor
		5-aza-2′-deoxycytidine		Pan inhibitor
		1-β-D-arabinofuranosyl-5-azacytosine		Pan inhibitor
		Dihydro-5-azacytidine		Pan inhibitor
		MG98		DNMT1

The current epigenetic modifying compounds are nonspecific and nonselective, acting both centrally and peripherally as well as at many epigenetic sites. The changes in the epigenome during the development of persistent pain are also extraordinarily complex and dynamic. Moreover, the resulting epigenetic mark largely depends on the injury or insult sustained as well as the past experiences of each individual. With this in mind, the current epigenetic drugs in development and in use for the treatment of cancer (Copeland et al., 2009; New et al., 2012) that may potentially be investigated in the context of pain are highly nonspecific HDAC inhibitors and DNMT inhibitors, which have numerous side effects and seem unsuitable for long-term treatment of chronic pain.