# 炎症与发育早期癫痫发生研究进展

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# 提纲

1. 癫痫及癫痫发作、分类及病因学
2. 未成熟大脑的癫痫发生：临床与基础研究
3. 炎症与癫痫易感
4. 治疗策略
5. 炎症介导的超兴奋机制

# 1、癫痫与癫痫发作

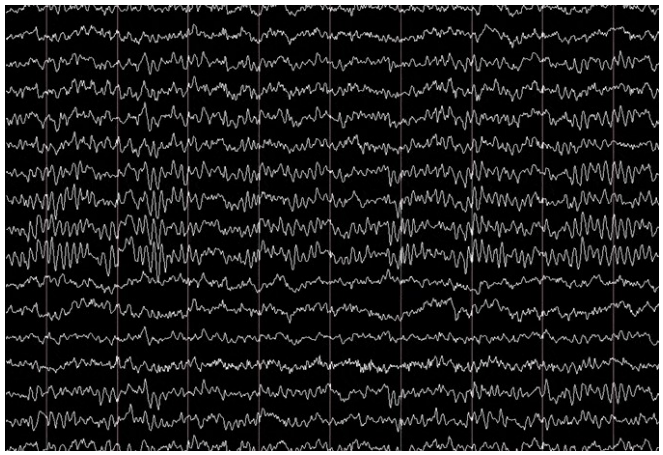
| | |
|---|---|
| 癫痫<br>Epilepsy | • 以持续存在的反复癫痫发作的易感性和由此引起的神经生物学、认知、心理学及社会方面后果的一种脑部疾病。 |
| 癫痫发作<br>Epileptic Seizures | • 是指脑部神经元异常过度放电引起的突然的、短暂的症状或体征（是一种临床症状） |
| 癫痫综合征<br>Epilepsy syndrome | • 在癫痫这一组疾病中,某些类型可以确定为独立的疾病类型,称之为癫痫综合征: West综合征, Lennox-Gastaut综合征 |

# 不同的年龄组常见病因

| | |
|---|---|
| 新生儿及婴儿期 | 先天以及围产期因素（缺氧、窒息、头颅产伤）、遗传代谢性疾病、皮质发育异常所致的畸形等 |
| 儿童以及青春期 | 特发性（与遗传因素有关）、先天以及围产期因素（缺氧、窒息、头颅产伤）、中枢神经系统感染、脑发育异常等 |
| 成人期 | 头颅外伤、脑肿瘤、中枢神经系统感染性因素等 |
| 老年期 | 脑血管意外、脑肿瘤、代谢性疾病、变性病等 |

# 1、癫痫与癫痫发作

## Neonatal Epileptic Seizures



•28 d (full term infants) or 44wk gestational age (preterm).

•the most frequent neurological problem

•electrographic seizure:

sudden, repetitive, evolving; >2 mV and 10s

## Main cause of seizure

•围产期窒息

•感染

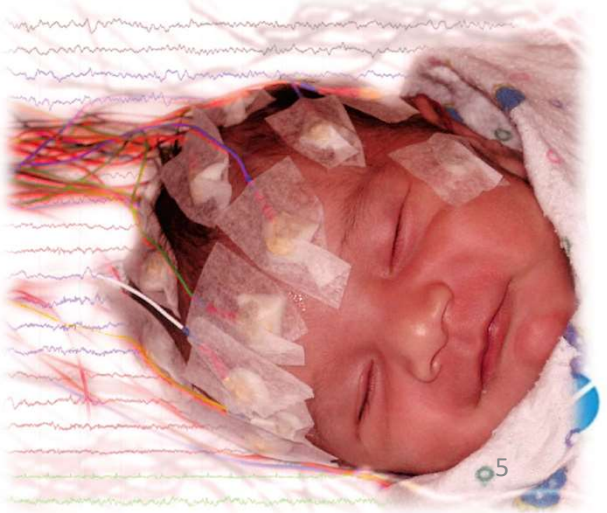
•代谢异常

•高热

## Main conflicting issues

•whether seizures in newborns can plant the roots for epileptogenesis

•cause long-term deficits

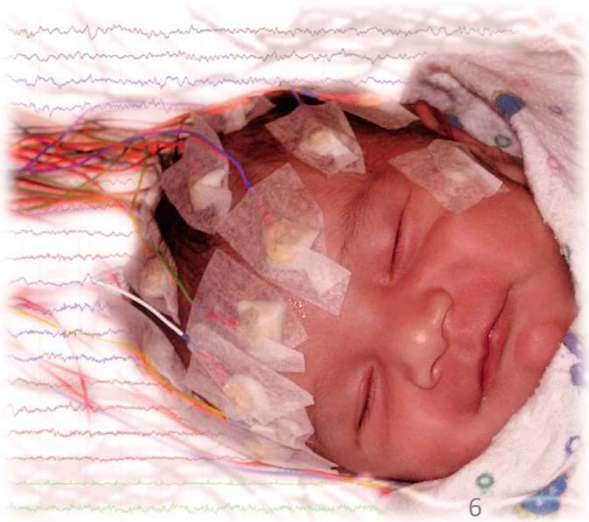


# 1、癫痫与癫痫发作

## Incidence of Neonatal Epileptic Seizures

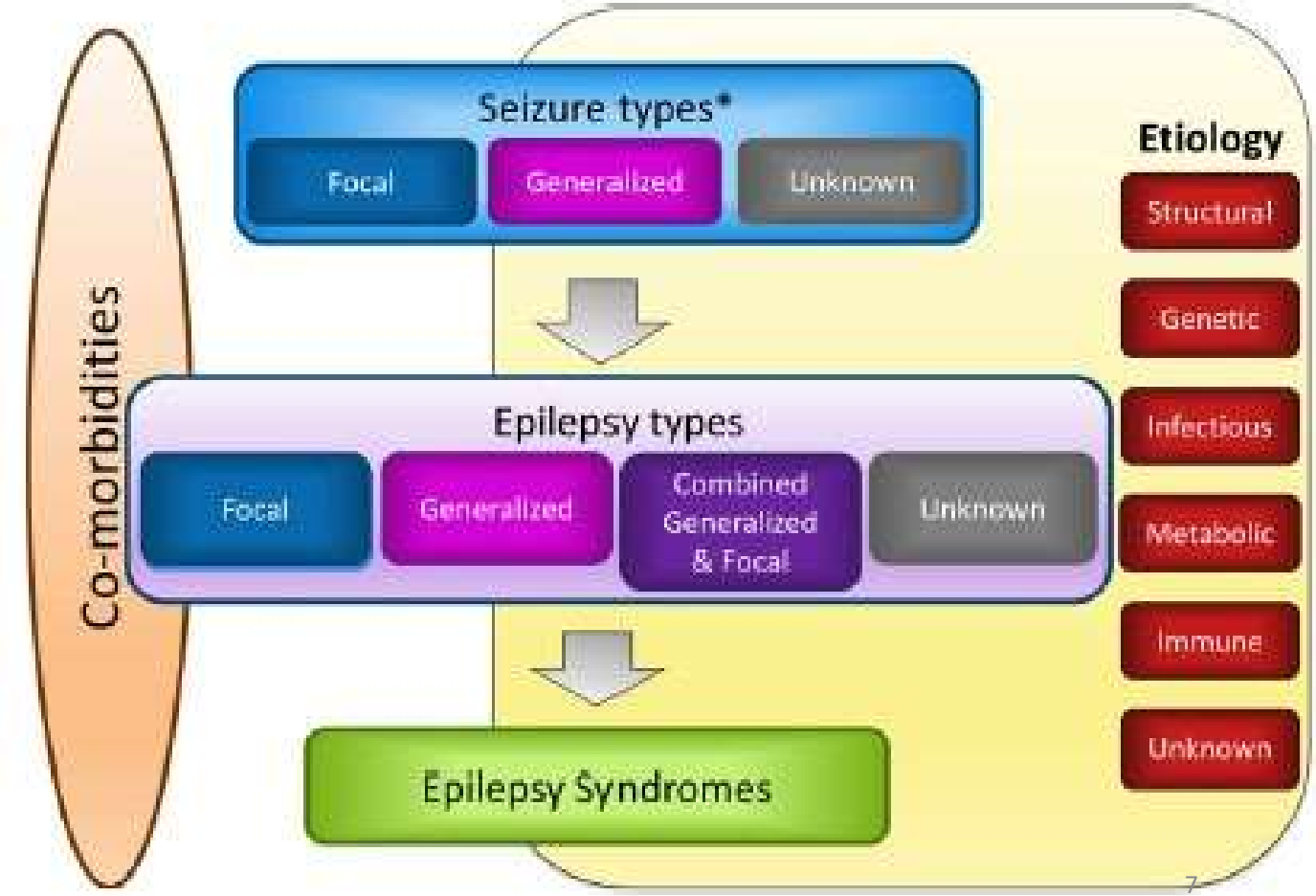
| Study | Seizure diagnosis | Incidence (/1000 LB) | VLBW (/1000 LB) | Type of study |
|---|---|---|---|---|
| Holden et al.[6] | Clinical | 5.0 | NA | Prospective |
| Lanska et al.[7] | Clinical | 3.5 | 57.5 | Retrospective |
| Lanska and Lanska[8] | Clinical | 2.4 | 9.4 | Retrospective |
| Saliba et al.[9] | Clinical | 1.8 | 19 | Retrospective/ prospective |
| Ronen et al.[10] | Clinical | 2.6 | 13.5 (<2500 g) | Prospective |
| Glass et al.[11] | Clinical | 0.95 | NA | Retrospective |

VLBW, very low birth weight; LB, live births.



# Classification 分类

国际抗癫痫联盟（ILAE）

# 2017年ILAE癫痫分类系统


## 离子通道功能异常

有缺陷的基因编码有缺陷的离子通道蛋白；

钠、钾、钙通道与癫痫

## 神经递质异常

●兴奋性与抑制性神经递质平衡，神经元膜稳定。

•兴奋与抑制间失衡，膜不稳定，产生癫痫性放电。

## 神经胶质细胞异常

神经元微环境的电解质平衡

神经胶质细胞对维持神经元的生存环境起着重要的作用。当星形胶质细胞对谷氨酸或γ氨基丁酸的摄取能力发生改变时可导致癫痫发作。

# Common Etiologies 病因学

## Metabolic

- Hypoglycemia
- Hypocalcemia
- Hypomagnesemia
- Hyponatremia
- Hypernatremia

- Maternal drug use – leading to withdrawal
- Inborn errors of metabolilsm (hyperammonemia, pyridoxine-responsive, hyperglycinemia)

## Cerebrovascular

- Hypoxic Ischemic Encephalopathy
- Arterial and Venous stroke
- Intracerebral hemorrhage
- Intraventricular hemorrhage
- Subdural hemorrhage
- Subarachnoid hemorrhage

## Infection

- Bacterial meningitis
- Viral meningitis
- Fetal infections
- TORCH infections

## Developmental

- Cortical dysplasia
- Schizencephaly
- Double cortex
- Lissencephaly

## Other

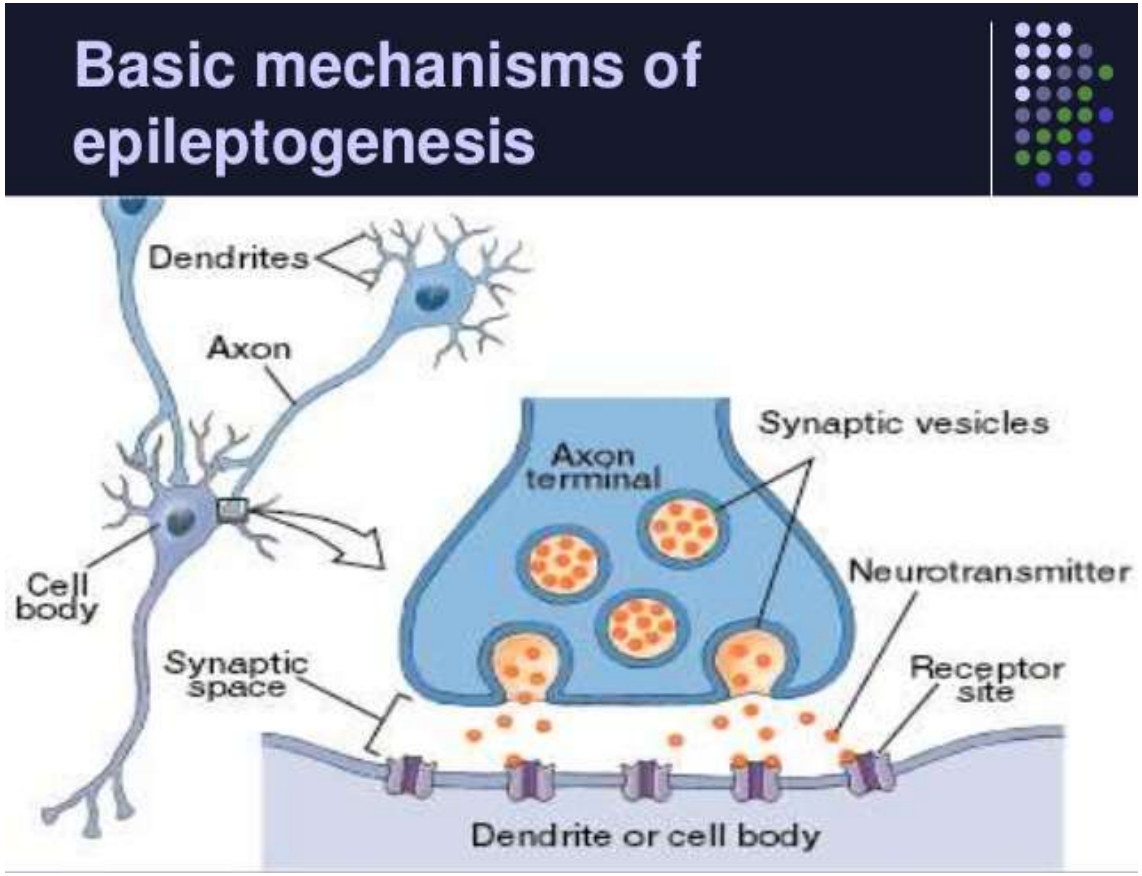
- Genetic disorders ( ARX, etc)
- Benign familial convulsions
- Early myoclonic convulsions

- Ohtahara syndrome
- Zellweger syndrome
- Pyridoxine deficiency

# Neonatal Epileptic Seizures

| Temporal Profile | Mechanism Targeted | Potential Therapeutic Options* |
|---|---|---|
| **Acute Changes** | Immediate Early Genes | Chromatin acetylation modifiers/histone deacetylation inhibitors (valproate) |
| | NMDA receptors | NMDA receptor inhibitors (memantine, felbamate) NR2B-specific inhibitors (Ifenprodil), |
| | AMPA receptors | AMPAR antagonists (topiramate, talampanel, GYKI compounds) |
| | NKCC1 Chloride Transporters | NKCC1 inhibitor (bumetanide -in combination with GABA agonists phenobarbital, benzodiazepines) |
| | GABA receptors | GABA receptor agonists (phenobarbital, benzodiazepines) |
| | Phosphatases (eg. calcineurin) | Phosphatase inhibitors (FK-506) |
| | Kinases (activation of PKA, PKC, CaMKII, Src kinases, etc) | Kinase inhibitors (CaMKII inhibitor KN-62, PKA inhibitor KT5720, PKC inhibitor chelerythrine) |
| **Sub-Acute Changes** | Inflammation | Anti-inflammatory compounds (ACTH), microglial inactivators (minocycline, doxycycline) |
| | Neuronal Injury | Erythropoietin, antioxidants, NO inhibitors, NMDAR antagonists (memantine) |
| | HCN Channels | I(h)-blocker ZD7288 |
| | CB1 receptor | CB1 receptor antagonists (SR 14176A, Rimonabant) |
| **Chronic Changes** | Sprouting | Protein synthesis inhibitors (rapamycin, cycloheximide) |
| | Gliosis | Anti-inflammatory agents, (Cox-2 inhibitors, minocycline, doxycycline) |

# 2、Epileptogenesis in neonatal brain 未成熟大脑的癫痫发生




动物实验与临床：

- Clinical evaluation?
- How to interpret brain developmental stages in rodents and compare them with humans?

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# Clinical evaluation 临床评价

- Physical Exam (including wood's lamp exam): Looking for both neurologic and systemic abnormalities
- History
- Imaging EEG
- Evaluation focused on looking for treatable causes, may be directed by history and exam
- Consider metabolic evaluation including:
  - Serology
  - Urine testing
  - Genetic testing
  - CSF testing

# 基础研究 brain developmental stages----rodents vs humans

Developmental stages in rodents based on maturation of hypothalamo-pituitary-gonadal axis

下丘脑-垂体-性腺轴

| Stage | Female (PN) | Male (PN) |
|---|---|---|
| Neonatal | 0–6 | 0–6 |
| Infantile | 7–21 | 7–21 |
| Juvenile | 21–32 | 21–35 |
| Early pubertal | 32–36 | 35–45 |
| Puberty | 34–38 | 45–60 |
| Adult | >60 | >60 |

PN, postnatal day.

Akman O, Moshe SL, Galanopoulou AS. Sex-specific consequences of early life seizures. Neurobiol Dis 2014

Species equivalency for developmental milestones

| Milestones | Rodents | Humans |
|---|---|---|
| Duration of gestation | 23 days | 40 weeks |
| Full-term neonate[a] | PN8–13 | 39–40 weeks |
| Eye opening | PN13–15 | Right after birth (~26 weeks) |
| Weaning from mother/end of breastfeeding | PN21 | 6th month or later |
| Ambulation | 2 weeks | >1st year of life |
| Life expectancy | 2 years | ~80 years in USA |

Galanopoulou AS, Moshe SL. In search of epilepsy biomarkers in the immature brain: goals, challenges and strategies. Biomarkers Med 2011

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# 3、Inflammation of epileptogenesis

- 危险因素（Infancy or adult）：

  CNS injuries：外伤 trauma，中风 stroke，病毒感染 viral infection，

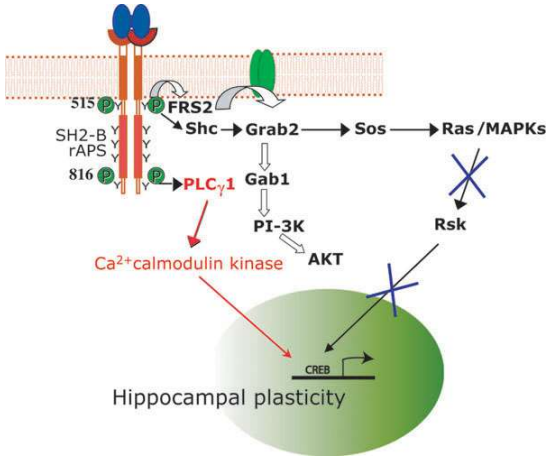
  热性惊厥 febrile seizures，癫痫持续状态 status epilepticus

- 现象：CNS inflammation立即发生，并长期存在
- 提示：pro-inflammatory state in the brain play a key role in the development of the epileptic process.
- 证据：

(1)the upregulation of pro-inflammatory signals during epileptogenesis in brain areas of seizure onset/generalization;

(2)pharmacological targeting of specific pro-inflammatory pathways after status epilepticus or in kindling shows antiepileptogenic effects.

# 3、Inflammation of epileptogenesis




➢机制：促炎因子利于长期-神经网络的超兴奋性(hyperexcitability)

(1)迅速-非转录效应： glutamate and GABA receptors

(2)基因转录激活：突触可塑性

➢基础研究策略：

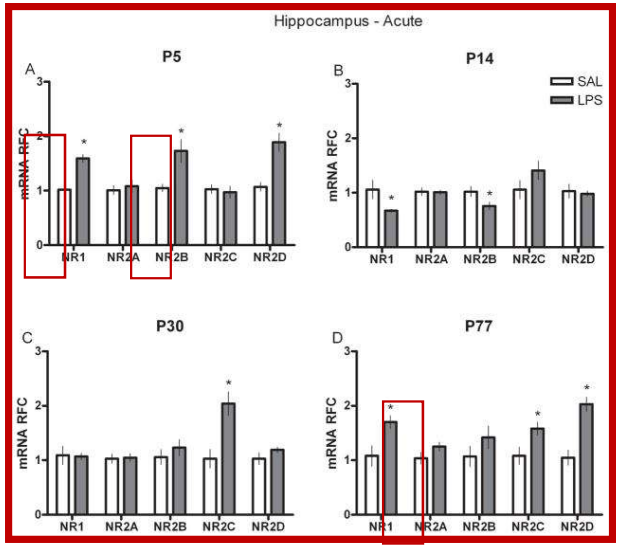
(1) 诱导CNS炎性状态，探讨短期/长期神经元兴奋性及发作阈值：administering pro-inflammatory molecules, or by using mice that overexpress specific cytokines

(2) 药理学干扰特异性炎症通路，效果评估：onset, phenotype, frequency and duration of spontaneous seizures.

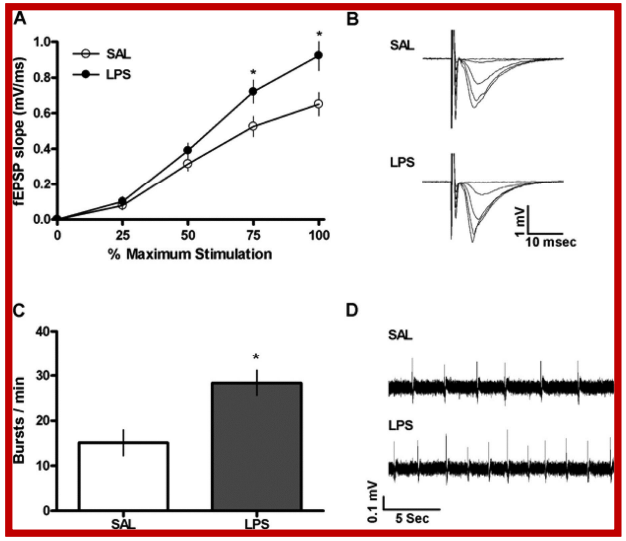
# 3-1 Inflammation and seizure susceptibility

- Immature rodents： PN7-14 days
- 模型: LPS or poly I:C (mimicking viral infection)—CNS inflammation and fever
- 易感性↓：未成年LPS→未成年高热诱导； 未成年LPS →成年匹鲁卡品诱导
- 神经细胞丢失↑； 认知缺陷↑： Microglia TNF- or IL-1 production ↑

早期炎症诱导海马谷氨酸受体亚型改变




内源性神经网络兴奋性改变



# Inflammation and seizure susceptibility

| Experimental model | Convulsant stimulus | Time of seizure test | Effect |
|---|---|---|---|
| ***Systemic infection*** | | | |
| Lipopolysaccharide in adult mice | PTZ | 1–12 h | ↓ seizure threshold |
| (Gram-negative) | PTZ | 24 h | ↑ seizure threshold |
| | Pilo in microglia/macrophage ablated animals | 24 h | ↑ seizure severity score |
| Lipopolysaccharide in PN6-PN14 rats | Li + Pilo, KA, PTZ<br>Febrile seizures | Adulthood | ↓ seizure threshold<br>↑ hippocampal excitability |
| *Shigella dysenteriae* sonicate in adult mice | PTZ | 7–24 h | ↑ seizure severity score |
| 2,4,6-Trinitrobenzene sulfonic acid (inflammatory colitis) in adult mice | PTZ | 2, 4, 10 days | ↓ seizure threshold |
| Mycobacterium adjuvant (arthritis) in adult rats | PTZ | 17 days | ↓ seizure threshold; ↑ seizure severity score |
| ***CNS viral infection*** | | | |
| Theiler's murine encephalomyelitis virus in adult mice | n.a. | 5–7 days | Occurrence of spontaneous seizures (50% of mice) |
| Herpes simplex virus type-1 in adult mice | PTZ | 15, 370 days | ↓ seizure threshold; ↑ seizure severity score |
| Polyinosinic:polycytidylic acid in PN14 rats | PTZ, Li + Pilo | Adulthood | ↓ seizure threshold |
| ***Traumatic brain injury*** | | | |
| Closed-skull impact model | Electroconvulsive shock | 7 days | ↓ seizure threshold |
| ***Transgenic mice*** | | | |
| Overexpressing TNF-α | KA | Adulthood | ↑ seizure severity score |
| Overexpressing IL-6 | KA, NMDA | Adulthood | ↑ lethality<br>↑ seizure severity score |

PTZ, pentylentetrazol; PN, post-natal day; KA, kainic acid; Li + Pilo, lithium + pilocarpine; n.a.: not applicable.

# 3-2 Enhanced potential for inflammatory response to seizures in the immature brain

1. HI及炎症大脑：早期的MG激活+炎症因子的产生
2. immature white matter：MG高表达（啮齿动物及人类)

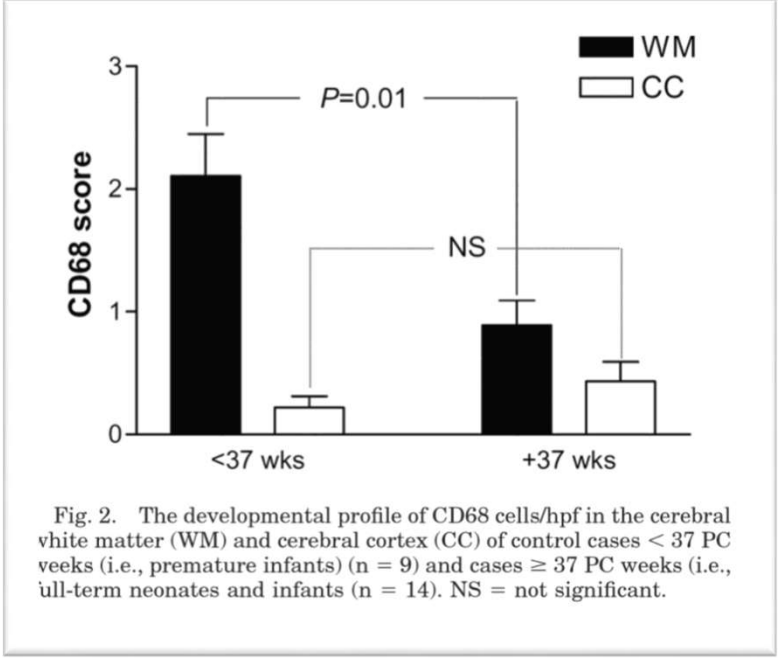



Fig. 2. The developmental profile of CD68 cells/hpf in the cerebral white matter (WM) and cerebral cortex (CC) of control cases < 37 PC weeks (i.e., premature infants) (n = 9) and cases ≥ 37 PC weeks (i.e., full-term neonates and infants (n = 14). NS = not significant.

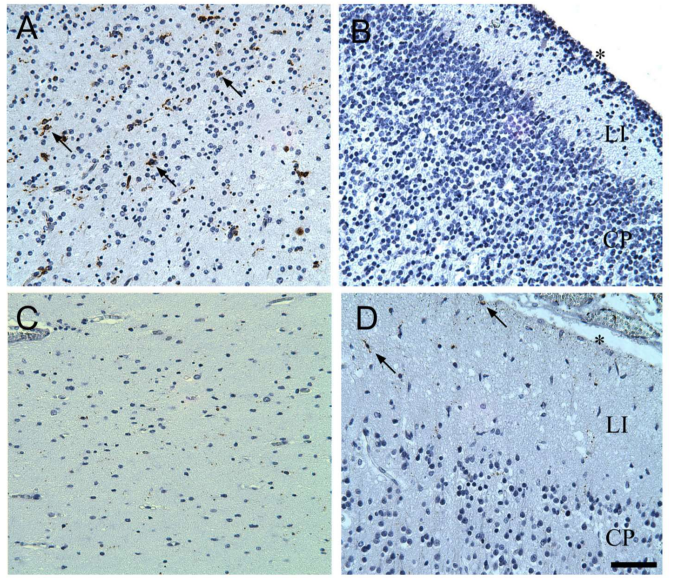
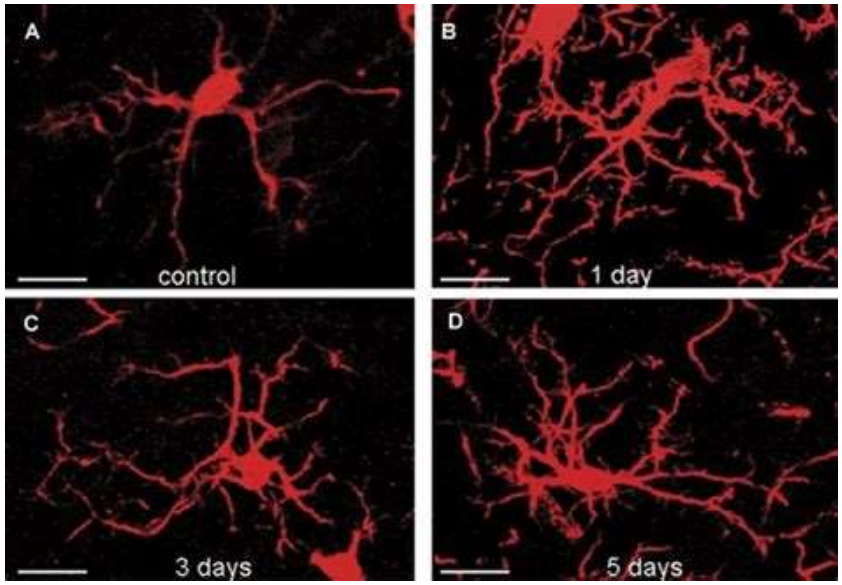



Fig. 3. Localization of activated microglia using CD68. **A:** Numerous activated microglia (arrows) in the white matter of a 23 PC week human fetus. **B:** Negligible CD68-immunopositive cells in the cortex of the same fetus. **C:** No CD68-immunopositive cells in the white matter of a 54 PC week human infant (14 postnatal weeks). **D:** Still low levels of activated microglia (arrows) in the cortex of the same infant. *Pia surface; LI, layer I; and CP, the cortical plate. All images are at 200× magnification. Scale bar = 60 μm (applies to all panels).

*Development of microglia in the cerebral white matter of the human fetus and infant*

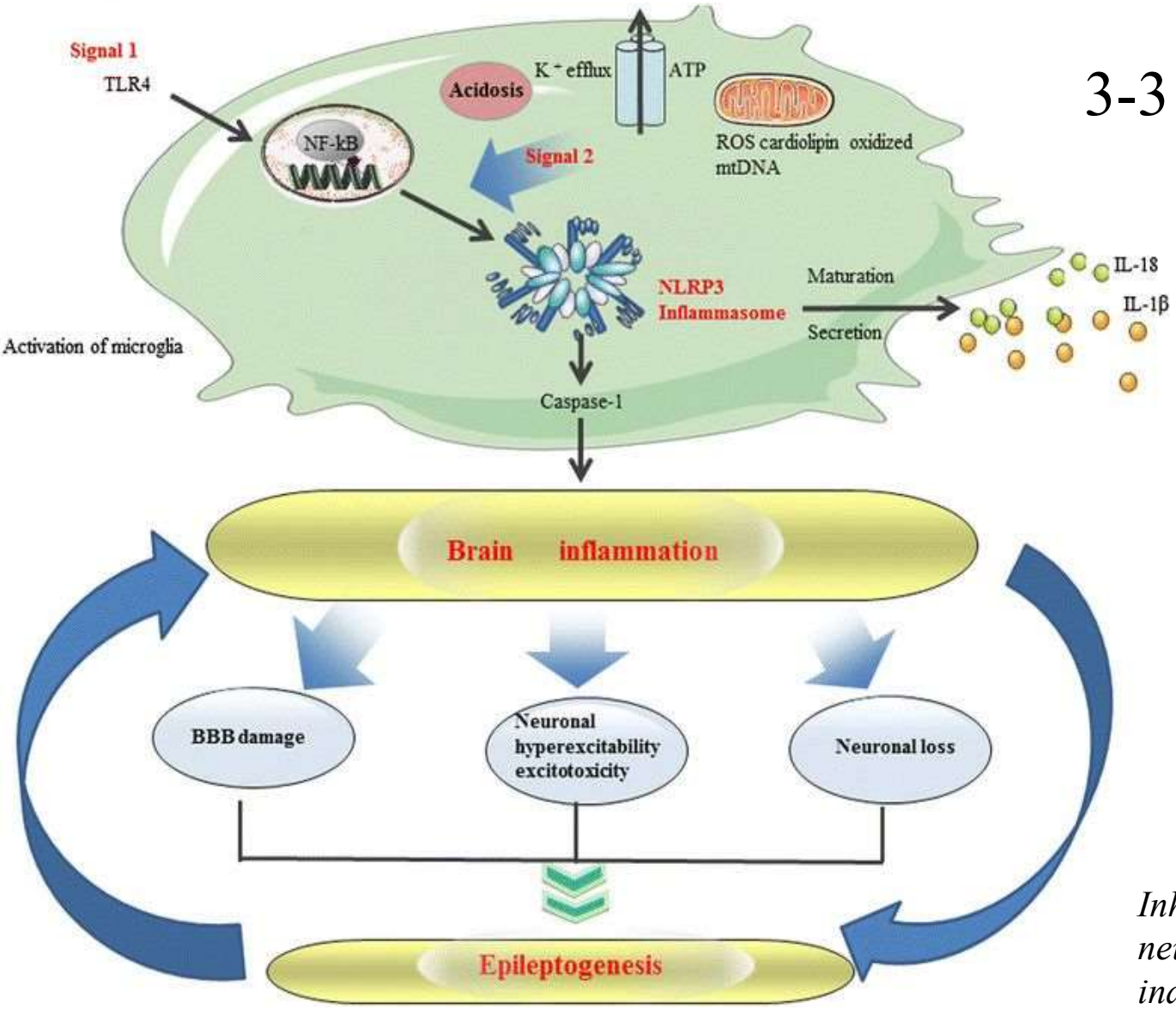
# 3-2 Enhanced potential for inflammatory response to seizures in the immature brain

3. 发育期：灰质深部的MG密度高于发育后期：细胞迁移有关
4. 不同癫痫模型诱导急性发作时：MG激活，形态学改变，迅速产生促炎细胞因子




*Rapid astrocyte and microglial activation following pilocarpineinduced seizures in rats. Epilepsia*

# 3-3 NLRP3 inflammasome

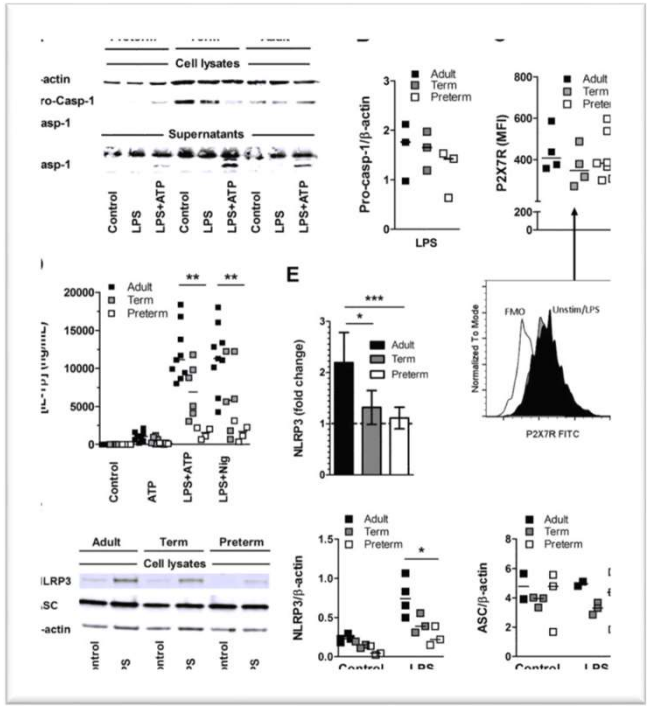


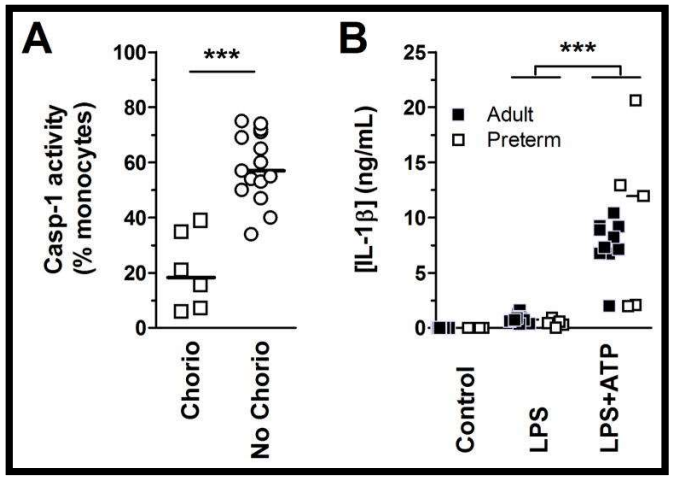

Preterm Adult

*Inhibition of the NLRP3 inflammasome provides neuroprotection in rats following amygdala kindling-induced status epilepticus*

# 3-3 NLRP3 inflammasome

- Impaired NLRP3 induction in preterm neonatal monocytes
- Developmental impairment in IL-1β secretion is restored after birth
- Such mechanisms may serve to limit potentially damaging inflammatory responses in a developing fetus




Preterm Adult

*Impaired NLRP3 inflammasome activity during fetal development regulates IL-1β production in human monocytes*

# 3-3 NLRP1 inflammasome

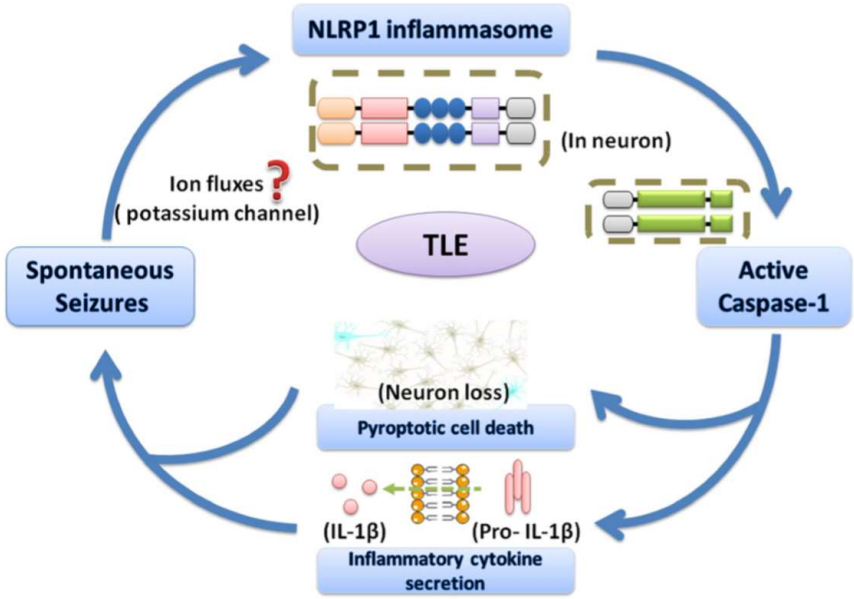
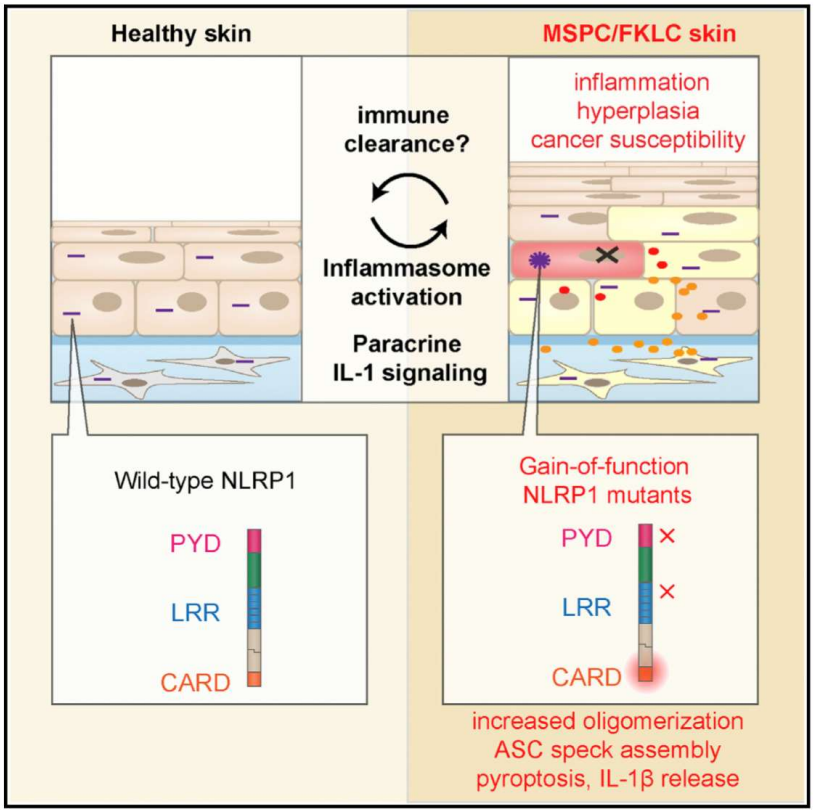



**Figure 4 NLRP1 inflammasome contributes to pyroptosis in chronic temporal lobe epilepsy.** High NLRP1 levels were found in pyramidal neurons of the brain. The spontaneous seizures may set fire to neuronal NLRP1 inflammasome via potassium efflux and other channels. Then, the activation of NLRP1 inflammasome leads to the caspase-1-mediated pyroptosis and secretion of IL-1β, which ultimately induces TLE pathology through several downstream effects in brain. Our current study mainly indicated that the caspase-1-induced neuronal pyroptosis provides a molecular basis for the spontaneous seizures in TLE process.

Preterm Adult

*NLRP1 inflammasome is activated in patients with medial temporal lobe epilepsy and contributes to neuronal pyroptosis in amygdala kindling-induced rat model*

# 3-3 NLRP1 inflammasome

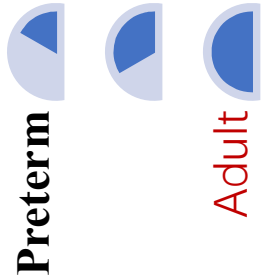



导致MSPC和FKLC的基

因突变破坏结构域，使得

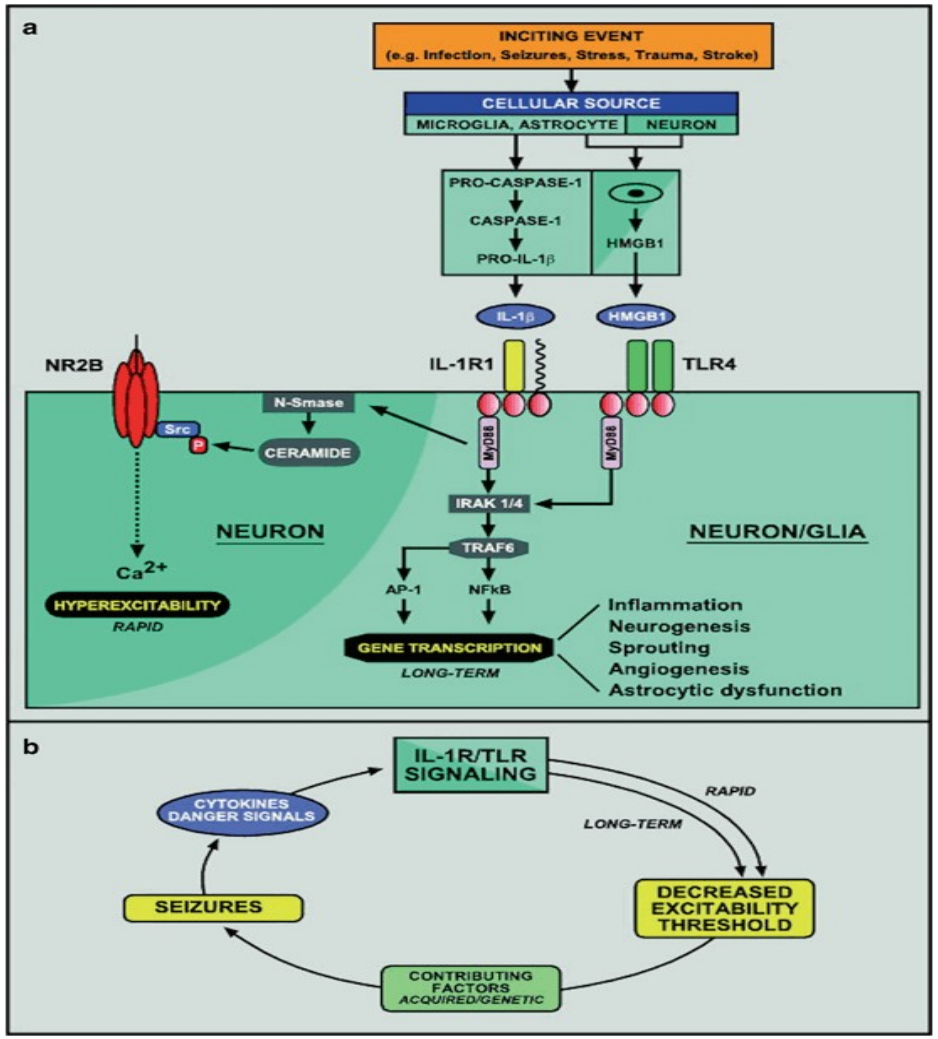
NLRP1更容易被激活，

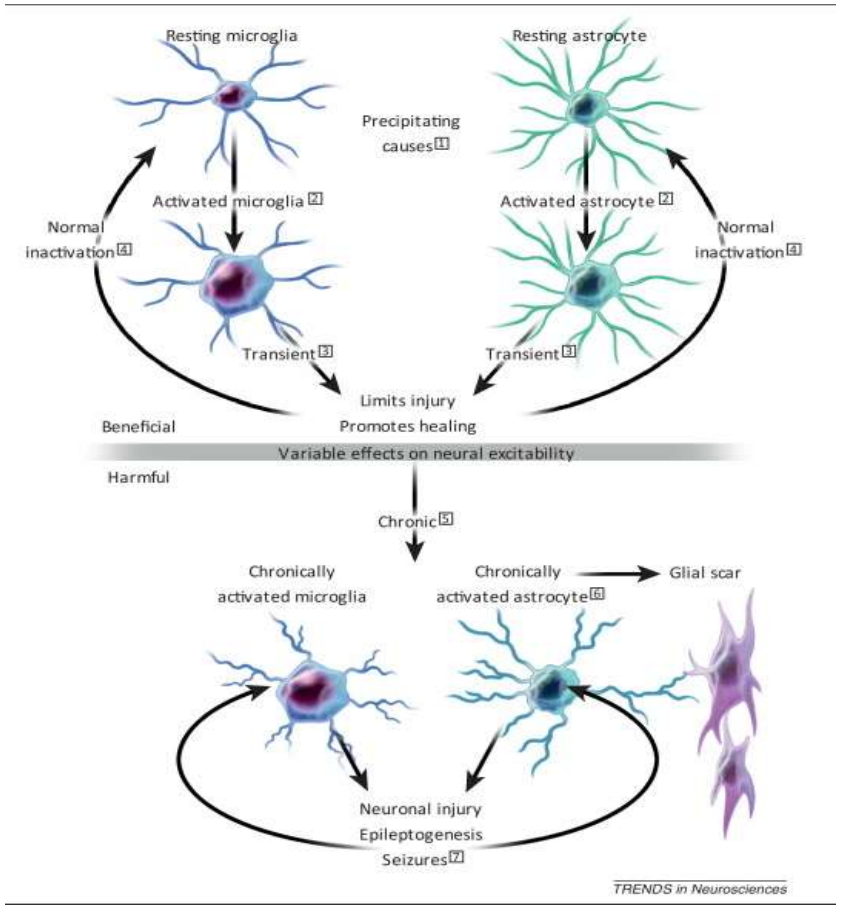
从而导致异常的炎症




*Germline NLRP1 Mutations Cause Skin Inflammatory and Cancer Susceptibility Syndromes via Inflammasome Activation. Cell, 167(1), 187–202.e17.*

# 3-4 Role of pro-inflammatory cytokines released from glia




*Crucial Role for Astrocytes in Epilepsy*

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# 4 Effects of anti-inflammatory treatments on epileptogenesis
## 针对炎症的治疗

1. 非甾体类Non steroidal anti-inflammatory drugs (NSAIDs)

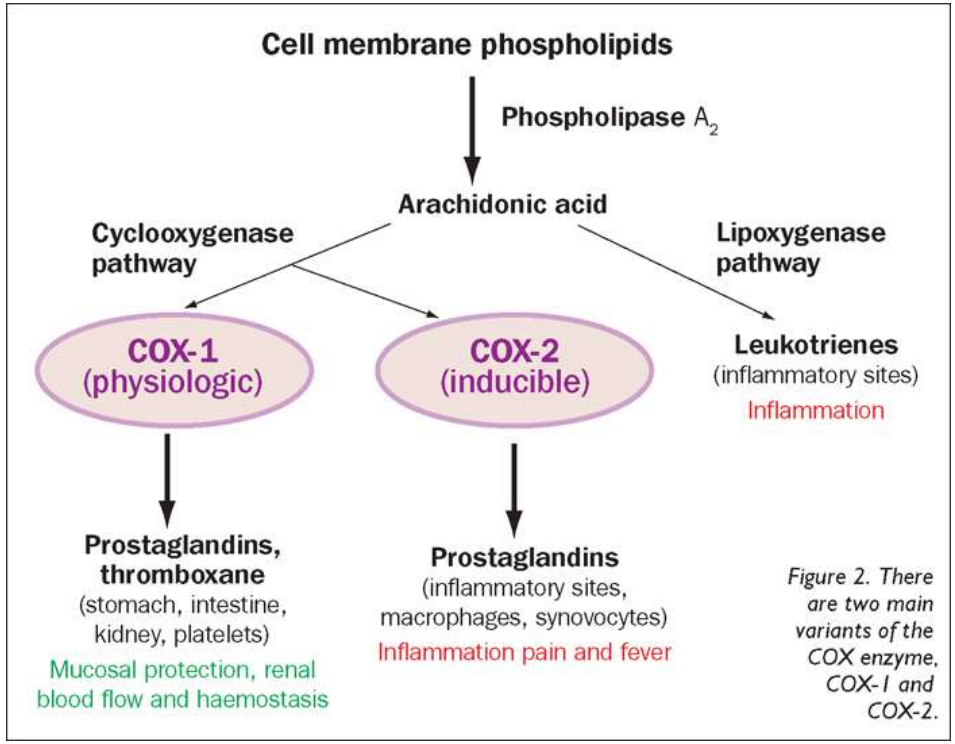

*Figure 2. There are two main variants of the COX enzyme, COX-1 and COX-2.*

- ➢ COX-2 选择性抑制剂，神经保护效应：
- celecoxib, parecoxib and SC58236
  --Status epilepticus-induced epileptogenesis
  --reduction in the number and frequency of video-monitored spontaneous seizures.

- ➢ 其他COX-2抑制剂nimesulide，rofecoxib
- ➢ 非选择性抑制剂paracetamol, naproxen, ibuprofen, mefenamic acid，indomethacin

- ➢ COX-1选择性抑制剂 SC560
  -- kindling model of epileptogenesis
  --反复PTZ注射或电刺激
  --delay in stage 5 seizure

# 4 Effects of anti-inflammatory treatments on epileptogenesis
## 针对炎症的治疗

1. 非甾体类Non steroidal anti-inflammatory drugs (NSAIDs)

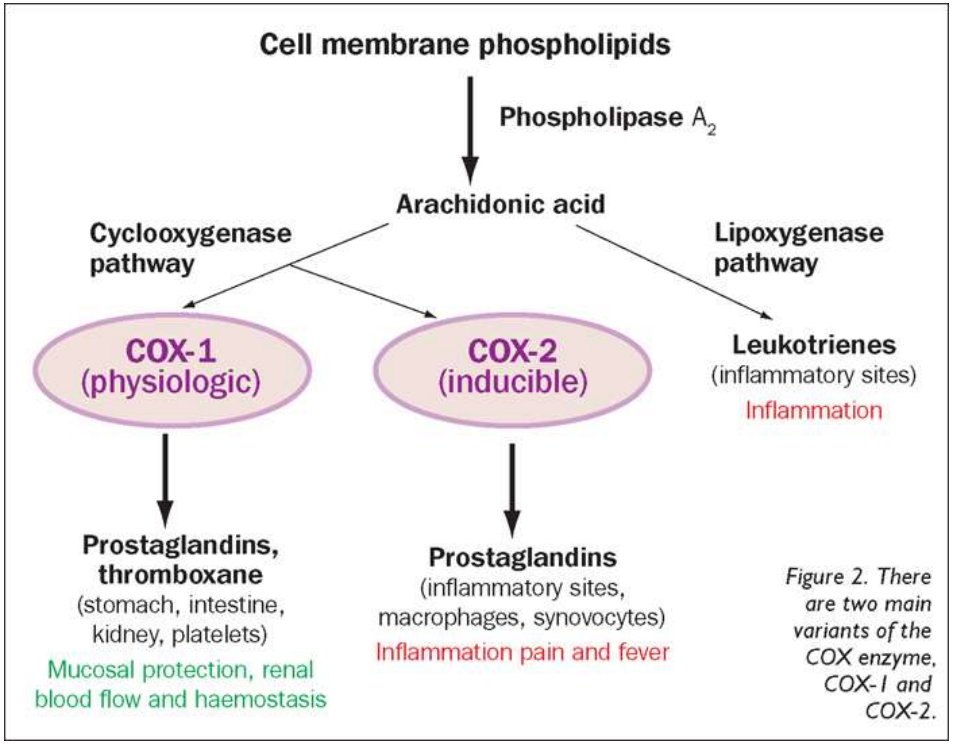

*Figure 2. There are two main variants of the COX enzyme, COX-1 and COX-2.*

- ➢ 下游PGE2信号
- 急性癫痫模型

  PGE2 促癫痫发生

  PGF2 抑制癫痫发生
- 提示：COX酶的作用依赖于不同模型中不同前列腺素的产生

# 4 Effects of anti-inflammatory treatments on epileptogenesis
## 针对炎症的治疗

2. 抗细胞因子Anti-cytokines approaches

| Drug | Experimental model | Treatment | Effect |
|---|---|---|---|
| IL-1β synthesis inhibitor | Electrical rapid kindling in rats | Every 90 min during stimulation | No kindling development |
| TNF-α | Electrical amygdala kindling in mice | 24 h before stimulation | ↑ AD duration; ↑ α activity |
| IL-10 | Electrical rapid kindling in rats | 1 h before stimulation | ↓ primary and secondary ADs; no effect on kindling development |
| Erythropoietin | Pilo-induced SE in rats | 24 h post-SE for 7 days | ↓ number and duration of spontaneous seizures |

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## 针对炎症的治疗

3. 免疫抑制剂Immunosuppressants

3种经典免疫抑制剂：

环孢霉素A cyclosporine A,

他克莫司FK-506 (Tacrolimus),

雷帕霉素rapamycin.

作用机制：

1. 抑制 T淋巴细胞激活
2. 雷帕霉素抑制mTOR激酶

| | | | |
|---|---|---|---|
| ***Immunosuppressants*** | | | |
| Rapamycin | KA-induced SE in rats | 24 h post-SE for 6 days | ↓ number of spontaneous seizures |
| Cyclosporin A | PTZ-induced kindling in mice | 1 h before PTZ | ↓ kinding development |
| | Electrical amygdala kindling in rats | 30 min before stimulation | ↓ kinding development |
| FK506 (Tacrolimus) | PTZ-induced kindling in mice | 1 h before PTZ | ↓ kinding development |
| | PTZ-induced kindling in rats | 30 min before PTZ | ↑ kindling development |
| | Electrical-induced SE in rats | 24 h post-SE for 14 days | No effects |
| | Electrical amygdala kindling in rats | 30 min before stimulation | ↓ kinding development |

# 提纲

1. 癫痫及癫痫发作、分类及病因学
2. 未成熟大脑的癫痫发生：临床与基础研究
3. 炎症与癫痫易感
4. 治疗策略
5. 炎症介导的超兴奋机制

# 5 Mechanisms of inflammation-mediated hyperexcitability
# 炎症介导的超兴奋机制

① Effects of pro-inflammatory cytokines on ion channels and receptors

- classic view：细胞因子通过自分泌/旁分泌方式诱导毒性介质生成
- Novel evidence：细胞因子通过调节神经元膜表面受体装配及磷酸化：

胶质细胞产生的IL-1 增加NMDA-介导的内向Ca2+ 电流， by activating IL-1R1 colocalized with NMDAreceptors on dendrites of pyramidal neurons

# 5 Mechanisms of inflammation-mediated hyperexcitability
# 炎症介导的超兴奋机制

① Effects of pro-inflammatory cytokines on ion channels and receptors

② Cytokines, PGE2 and extracellular glutamate

- TNF诱导的astrocytes 释放 PGE2，介导Ca2+依赖的谷氨酸释放

# 5 Mechanisms of inflammation-mediated hyperexcitability
# 炎症介导的超兴奋机制

① Effects of pro-inflammatory cytokines on ion channels and receptors

② Cytokines, PGE2 and extracellular glutamate

③ COX-2 and PGE2

CA1神经元中，PGE2通过减少钾电流， 增加frequency of firing and EPSP amplitude

# 5 Mechanisms of inflammation-mediated hyperexcitability
## 炎症介导的超兴奋机制

① Effects of pro-inflammatory cytokines on ion channels and receptors

② Cytokines, PGE2 and extracellular glutamate

③ COX-2 and PGE2

④ Cytokines and BBB

IL-1ß and TNF-α 增加血管通透性，促进血管生成；

血管周的astrocytes 改变BBB， 提高周围神经元兴奋性

# 5 Mechanisms of inflammation-mediated hyperexcitability
# 炎症介导的超兴奋机制

① Effects of pro-inflammatory cytokines on ion channels and receptors

② Cytokines, PGE2 and extracellular glutamate

③ COX-2 and PGE2

④ Cytokines and BBB

⑤ Long-term transcriptional effects

炎性介质介导细胞死亡、神经发生及突触重构；

# 小结

1. 病因：离子通道异常、神经递质异常，胶质细胞功能异常
2. 促炎因子利于神经网络的超兴奋性，机制是：ion channels and receptors，transmitter, BBB
3. MG发育特征、NLRP3发育特征
4. 治疗炎症，控制癫痫发生

***Thank You***