in a Cbfa1$^{+/-}$ background maintained their osteosclerotic phenotype[1].

A second model suggests that an excess of Fra1 or ΔFosB molecules could heterodimerize with, and titer out, a transcriptional inhibitor of osteoblast differentiation (Fig. 1*b*). Because Fra1 and ΔFosB are not required for osteoblast differentiation *in vivo*[1,6], this type of dominant-negative interaction could occur only if Fra1 and ΔFosB heterodimerize in the transgenic animals with a protein with which they do not heterodimerize under physiological circumstances. As the authors of both reports acknowledge, there is currently not enough evidence to support either of these models.

Through the use of the gene deletion technology, a wide array of molecules has been identified that regulates bone remodeling. Many of these molecules are unexpected, ranging from hormones such as leptin[7] to tyrosine kinases such c-src and c-abl (refs. 8,9) to transcription factors such as Pu-1, c-fos, NF-κB, Msx2, Cbfa1 (ref. 8). Moreover, overexpression studies such as those by Jochum, *et al.*[1] and Sabatakos, *et al.*[2] show that even molecules that normally do not control osteoblast differentiation can, under certain conditions, tilt the precarious equilibrium of bone remodeling toward a disease state.

Analysis of various animal models has made it clear that uncontrolled bone formation eventually leads to extramedullary hematopoiesis, anemia and early death. Likewise, a failure to resorb bone properly also causes diseases such as osteopetrosis. Bone remodeling, like most other homeostatic functions, is a highly complex process, and improper regulation can lead to many different types of diseases, explaining why the list of regulators is so long. Given the new questions raised by these two important mouse models, and by others, it is safe to assume that the list is not complete yet.

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Pain, plasticity, and premature birth: a prescription for permanent suffering?

A collection of clinical and animal studies suggest that exposure to pain during the neonatal period leads to long-term changes in neural circuitry and behavior, contradicting the theory that infants don't 'remember' painful experiences.

K.J.S. ANAND

ABOUT 11,000 NEWBORN infants are receiving intensive care in the U.S. today, and many of these will be exposed to multiple painful invasive procedures[1–3]. In contrast to similar invasive procedures performed in the adult intensive care unit (ICU), few of these neonates will receive analgesic therapy in preparation for tissue damaging procedures[1–3]. An inflammatory response lasting from hours to days will follow, leading to increased pain sensitivity (hyperalgesia) around the area of damaged tissue. This inflammatory response usually goes untreated. It is commonly believed that "babies can't remember these painful experiences". However, recent studies suggest that although early painful memories are not accessible to conscious recall, they may be encoded in "procedural memory" and lead to abnormal behavioral patterns or altered sensory processing in later life[4,5]. In a recent issue of *Science*, Ruda *et al.* [6] made the startling discovery that localized inflammation during the neonatal period *permanently* alters neuronal circuits that process pain in the spinal cord.

In an elegant series of experiments, these researchers injected complete Freund's adjuvant into the left hindpaw of 0, 1, or 3 day-old rat pups to produce localized inflammation that lasted for 5 to 7 days. When these animals reached adulthood, they had increased numbers of primary sensory (afferent) fibers exiting the sciatic nerve, and these fibers connected with superficial layers of the spinal cord dorsal horn that are involved in pain processing (laminae I / II, called the substantia gelatinosa). These thinly myelinated or unmyelinated nerve fibers also extended into caudal segments of the spinal cord (L6, S1) that don't normally receive sensory input from the sciatic nerve. Dorsal horn neurons receiving input from these terminals were hyperexcitable, demonstrating increased activity at rest and in response to tactile or noxious stimuli. This led to an increase in pain response behaviors in adult rats[6].

Thus, neonatal painful experiences may leave a legacy of altered sensitivity to subsequent pain, a concept proposed 13 years ago[7]. For example, neonatal rats exposed to four daily needle sticks during the first week of life were more sensitive to pain at 16 and 22 days of age[8]. Other studies reported that excessive sprouting of cutaneous nerve fibers occurs following neonatal skin wounds, associated with lowered thresholds for the withdrawal reflex for several weeks following the injury[9]. The excessive nerve sprouting was inhibited by antibodies against nerve growth factor (NGF) in adult but not neonatal rats, suggesting that more than one growth factor induces nerve growth during this developmental time period[10]. The cutaneous nerve sprouting in neonates was not inhibited by sciatic nerve blockade, suggesting that nerve fiber function is not required for this sprouting to occur[11].

Another study reported that injection of carrageenan into the hindpaws of 1 day-old rats[12] led to significant (33%) reductions in the receptive field size (area of skin from which a dorsal horn neuron receives sensory stimuli) of spinal cord sensory neurons in adult rats. Collectively, the long-term effects of neonatal pain include hyperinnervation in areas of wounded skin[9], with increased sprouting of primary afferent fibers and hyperexcitability of sensory neurons[6]. The enhanced sensory input from "hypersensitive" skin may lead to adaptive changes within the spinal cord dorsal horn that decrease the receptive field size of afferent neurons[12]. The reduction in receptive field size may be caused by reduced dendritic arborization of afferent neurons, increased connections with inhibitory interneurons, up-regulation of inhibitory receptors or ion channels, or enhanced activity of the descending inhibitory controls from supraspinal centers[13].

Similar results have also been seen in infants. Clinical data suggest that the physiological or behavioral responses to pain are altered by exposure to repetitive or prolonged pain during early development[4,5,14]. For example, healthy infants undergoing routine vaccination at 4-6 months of age were noted to have accentuated behavioral responses (measured by facial expressions, duration of crying, and visual analogue scales by blinded observers) if they were exposed to unanesthetized circumcision at birth, as compared with uncircumcised infants or those receiving topical anesthesia before circumcision[15].

However, in evaluating the clinical relevance of these findings, Ruda *et al.* must consider the large differences in complexity and adaptability between human and rodent brains, and also developmental differences between neonatal rats and humans. The authors' experimental system involved induction of localized inflammation for 5-7 days in neonatal rats. In humans, this would correspond to inducing several weeks of continued pain during late gestation, which rarely occurs in the clinical setting. Also, such an inflammatory response would be expected to cause localized fibrosis, limb deformities, and other trophic changes, which may contribute to the spinal cord changes reported by Ruda *et al.* Injection of complete Freund's adjuvant also induces a systemic inflammatory response leading to generalized arthritis and spinal cord changes [16], which may have contributed to these findings.

Studies on preterm neonates that spent 4 weeks in the neonatal ICU have also reported dampened behavioral responses to painful procedures, such as to the heel lance required for blood sampling. Dampened behavioral responses were correlated with the number of painful procedures that the neonates received[17]. Measurements of facial behavioral and cardiac autonomic reactivity during a finger-lance blood collection showed similar results in groups of 4 month-old infants who were born either preterm or full-term[18]. Similarly, 18 month-old ex-preterm infants were less sensitive to everyday pain when compared with matched controls who were born full-term[19]. The greater the number of painful procedures infants had experienced as neonates, the less responsive they were to pain as toddlers. Altered behavior was also seen in older children that had spent time in the neonatal ICU as infants[20,21]. These studies suggest that painful experiences in late human gestation seem to enhance, whereas painful experiences in early human gestation seem to dampen the behavioral responses to subsequent pain.

Given the plasticity of supraspinal foci involved in sensory processing, repetitive pain during infancy may cause widespread changes in the immature brain leading to abnormal behaviors in adulthood. Exposure of neonatal rat pups to daily needle-stick pain led to decreased pain thresholds in late infancy, along with increased alcohol preference and anxiety-mediated behaviors during adulthood. These behavioral changes resemble some of the behaviors seen in older children that were born prematurely and exposed to painful procedures in the neonatal ICU[8].

Across the different laboratory models, it is becoming increasingly clear that experiences of pain will be "remembered" by the developing nervous system, perhaps for the entire life of the individual. These findings should focus the attention of clinicians on the long-term impact of early painful experiences, and highlight the urgent need for developing therapeutic strategies for the management of neonatal pain. Otherwise, future generations will pay the price for medical procedures performed today[22].

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Ibuprofen, inflammation and Alzheimer disease

Mounting evidence suggests that non-steroidal anti-inflammatory drugs may be useful in reducing the risk of Alzheimer disease. After much study, the mechanism by which these drugs reduce the amyloid deposition associated with the disease is still open to speculation.

TONY WYSS-CORAY & LENNART MUCKE

NON-STEROIDAL ANTI-INFLAMMATORY drugs (NSAIDs) such as aspirin or ibuprofen are widely used to alleviate fever, pain and inflammation. Because they also inhibit platelet aggregation, NSAIDs are also used to prevent myocardial infarction and stroke. Epidemiological studies suggest that the use of NSAIDs may reduce the risk of developing Alzheimer disease (AD), and clinical trials are underway to test their efficacy in the treatment of AD (ref. 1). A study, recently published by Lim *et al.*[2] in *The Journal of Neuroscience*, lends new experimental support to the idea that NSAIDs may be useful for the treatment of AD. The investigators tested the effects of orally administered ibuprofen, a commonly used NSAID, in a transgenic mouse model of AD. After 6 months of treatment, the mice showed less AD-type pathology than untreated controls.

AD is the most common form of dementia. The relentless cognitive decline in AD patients is associated with the abnormal accumulation of amyloid deposits (plaques) in the brain. Amyloid proteins are a group of diverse proteins that have a tendency to form aggregates[3]. Different types of amyloid aggregates share histologic staining characteristics, and depending on the organ in which they accumulate, cause a variety of diseases. Organs can be affected by primary amyloidoses as well as by secondary amyloidoses that result from chronic inflammation induced by other diseases[3]. The findings of Lim *et al.* suggest that molecular targets of NSAIDs play a key role in the development of brain amyloidosis[2], an important element, if not the main cause, of AD (refs. 4–6).

The amyloid protein that is deposited in AD brains is the Aβ peptide, which is derived from the larger amyloid protein precursor (APP) (refs. 4–6). APP mutations that increase the production of fibrillogenic Aβ peptides cause rare forms of autosomal dominant familial AD. Neuronal expression of mutant APP elicits various AD-like pathological alterations in transgenic mice, including amyloid plaques and distortions of neuronal processes (neuritic dystrophy). The plaques in the brains of AD patients and APP mice trigger reactions of local astrocytes and microglia (gliosis). These injury-responsive cells produce a large number of inflammatory mediators including cytokines such as interleukin (IL)-1. The gliosis observed in AD patients and the reduced incidence of AD in people taking NSAIDs raise the question of whether inflammatory processes play a prominent role in the pathogenesis of AD (ref. 1). If so, anti-inflammatory drugs such as NSAIDs might prevent or reduce AD-like pathology.

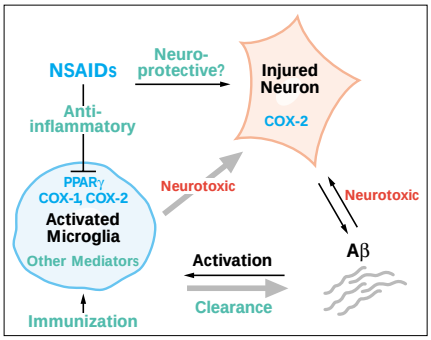

Fig. 1 Potential effects of ibuprofen on AD-type pathology. The Aβ peptide (gray wavy lines) is produced predominantly by neurons and, when aggregated, can injure neurons and activate microglia. Microglia (circle), the main phagocytic cells of the brain, may play a key role in the clearance of diffusible and plaque-deposited Aβ aggregates. The aberrant activation of specific microglial pathways, however, could also induce neurotoxic effects. Similar dual effects can also be found in other diseases involving a chronic inflammatory response. NSAIDs inhibit cyclooxygenase (COX) -1 and COX-2 production, and activate peroxisome proliferator-activated receptor-γ (PPAR-γ). Inhibition of neuronal COX-2 could have direct neuroprotective effects on neurons, and might also alter Aβ production. Alternatively, NSAIDs may act through microglia, inhibiting COX enzymes and activating PPAR-γ to reduce the production of neurotoxic agents. Immunization with Aβ and other unknown effects of NSAIDs or endogenous factors may activate alternative microglial pathways that prevent or reverse the accumulation of Aβ in the brain.

Lim *et al.* tested this hypothesis by treating APP transgenic mice with ibuprofen[2]. Treatment was initiated when the mice were 10 months old, the age at which plaque deposition typically begins in this model. The brains of treated and untreated mice were compared 6 months later. The treated mice showed significant reductions in cerebral plaque load, soluble and insoluble Aβ, neuritic dystrophy, plaque-associated gliosis and IL-1 concentrations.

Although the study by Lim *et al.* was not designed to determine the mechanism of ibuprofen action or the relationship between inflammatory changes and cognitive deficits, the findings, along with the ongoing clinical trials of NSAIDs for AD, make it interesting to speculate about these issues. Three molecular targets of NSAIDs have been identified as potential mediators of their anti-inflammatory actions. NSAIDs inhibit the constitutively expressed cyclooxygenase (COX-1) and the inducible form of this enzyme (COX-2), both of which produce prostaglandins[7]. NSAIDs also activate the nuclear peroxi-