The importance of ‘awareness’ for understanding fetal pain

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Abstract

Our understanding of when the fetus can experience pain has been largely shaped by neuroanatomy. However, completion of the cortical nociceptive connections just after mid-gestation is only one part of the story. In addition to critically reviewing evidence for whether the fetus is ever awake or aware, and thus able to truly experience pain, we examine the role of endogenous neuroinhibitors, such as adenosine and pregnanolone, produced within the feto-placental unit that contribute to fetal sleep states, and thus mediate suppression of fetal awareness. The uncritical view that the nature of presumed fetal pain perception can be assessed by reference to the prematurely born infant is challenged. Rigorously controlled studies of invasive procedures and analgesia in the fetus are required to clarify the impact of fetal nociception on postnatal pain sensitivity and neural development, and the potential benefits or harm of using analgesia in this unique setting.

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1. Introduction

Whether the fetus can truly experience pain, at least in some way analogous to how adults emotionally understand pain, has been debated extensively over recent years and is of importance given continuing advances in fetal surgical and diagnostic procedures [43]. This question has considerable implications for the management of invasive fetal procedures [16,78,94], particularly as fetal analgesic and anaesthetic treatment is complex [195] and not without risk for the fetus [171]. Prevention and treatment of pain are basic human rights, regardless of age, and if fetal interventions are to progress, then a greater understanding of nociception and stress responses is required [236].

The timing of the neuroanatomical maturation of the nociceptive system is now well understood, and the final critical cortico-thalamic connections appear to be present by 24–28 weeks of gestation [25,40,56,78,136,236]. This suggests that the fetus could potentially be able to feel pain by the third trimester, at least in a rudimentary fashion. This concept is said to be supported by studies which show that nociceptive stimuli elicit physiological stress-like responses in the human fetus in utero [210].

However, physiological processing of a nociceptive stimulus and perceiving a nociceptive stimulus as painful are not the same. There are both a physiological and an emotional or cognitive aspect to pain perception, and indeed a significant element of learning [56]. Certainly, processing can be independent of perception, as is demonstrated during surgery under general anesthesia, for example, where nociceptive stimuli can still elicit subcortically mediated physiological stress responses despite unconsciousness [57,85,140]. Thus, to emotionally experience pain, we must be cognitively aware of the stimulus (a cortical process), and this in turn requires that we must be conscious [25,40,56].

The key question then is not about the anatomic completion or functionallity of nociceptive pathways in utero, but whether the fetus is ever conscious and thus aware. In general, discussion of fetal pain perception tends to treat the fetus as an unborn newborn; i.e., that responses of the newborn represent an adequate surrogate for the fetus. The assumption is thus made that if the newborn (including the preterm newborn) can experience wakefulness (and therefore consciousness), and apparently feels pain, then so too must the age-equivalent fetus. Furthermore, evidence for fetal wakefulness (and again therefore consciousness) has been based on how certain fetal responses “resemble” newborn sleep–wake behaviors, rather than a true determination of fetal wakefulness per se. Given the complexities of studying the fetus, extrapolation from or to the newborn state is understandable. Systematic studies of fetal neurological function suggest, however, that there are major differences in the in utero environment and fetal neural state that make it likely that this assumption is substantially incorrect. This has important implications for our understanding of fetal pain perception.

The current review critically evaluates the hypothesis that unlike the newborn, the fetus is actively maintained asleep (and unconscious) throughout gestation and cannot be woken by nociceptive stimuli. The evidence is examined with reference to fetal sleep–wake states, the role of cortico-thalamic gating in cortical arousal during sleep, and the unique contribution that certain inhibitory neuromodulators make in utero to cortical suppression. Finally, we briefly discuss the validity of the hypothesis that suggests that the nociceptive input may have long-lasting deleterious effects regardless of whether the fetus is asleep or not.

2. Fetal responses to nociceptive stimuli

2.1. Neuroanatomical maturation

The processing of nociceptive stimuli requires peripheral sensory receptors, afferent and efferent sensory and motor pathways, and subcortical and cortical neural integration of the related impulse traffic [8]. The development of nociceptive pathways has been extensively reviewed by others and is not the subject of this review [13,15,56,64,66,118,174,230,236]. In brief, however, it is generally agreed that an integrated pathway exists by 24–28 weeks of gestation and that it includes the critical cortico-thalamic connections deemed to be essential for the experience of pain [78,136]. The thalamus is an obligatory station through which nearly all sensory information must pass before reaching the cerebral cortex. One major function of the thalamus is the selective control of the flow of sensory-
motor information to the cerebral cortex during different states of the sleep–wake cycle and arousal, modulated by inputs from the brainstem, hypothalamus, and cerebral cortex [146].

Several commentaries suggest that once the nociceptive pathway is complete the fetus may experience pain and that various behavioral and physiological responses may reveal fetal awareness or subjective consciousness of pain [25,78,210]. For instance, the human fetus responds to intrahepatic needling (versus umbilical cord sampling) by moving away and with an increase in the levels of circulating stress hormones such as cortisol, corticotropin releasing hormone (CRH), catecholamines, and beta-endorphins [71,72,74,75]. These responses are independent of maternal responses [72,74]. Moreover, fetal intrahepatic sampling leads to a redistribution of the combined ventricular output away from peripheral organs such as the gut and kidneys towards central organs such as the heart and brain [211,224], and scalp sampling increases heart rate in some fetuses [215].

2.2. The question of cortical awareness

It is noteworthy, however, that these responses are elicited at the subcortical and brainstem level and do not require cortical input [78,136,243]. Thus, they cannot be said to represent evidence for cortical awareness. For example, very similar cardiovascular redistribution responses are seen during severe experimental fetal hypoxemia as part of a coordinated fetal defense, and occur even when neural activity is essentially isoelectric; i.e., when fetal brain activity is profoundly suppressed [30,76,107,112,115]. Consistent with this, stress hormones are significantly elevated during surgical procedures carried out under general anesthesia [57,85,140] and in brain dead patients during organ harvesting [68,137]. Indeed, even an anencephalic fetus responds to somatic stimulation by withdrawal, demonstrating that these reflexes are mediated entirely at a subcortical level [233].

Similarly, patients in vegetative states are thought to be incapable of conscious experience themselves, even though they are able to be aroused [242], and in such patients flexor withdrawal and other reflexes, which are mediated through the thalamus, are preserved [179]. In the condition of sleepwalking, motor arousal can clearly occur, but while sleep is disturbed it is not to the extent of full awakening [5]. Similarly, in the neonate, arousal-eliciting stimuli can also cause respiratory and motor responses without necessarily leading to cortical arousal [149]. Likewise, moderate pain can elicit physiological and behavioral responses during sleep in adults without the subject arousing or waking [69]. These variable cortical states demonstrate that hormonal and arousal responses to noxious stimuli can be elicited independent of cortical awareness. This, however, does not mean that fetal awareness does not occur. The question is then, is the fetus ever conscious?

2.3. Is the fetus ever awake?

2.3.1. Characterization of wakefulness

The existence of consciousness is, of course, difficult to establish in the fetus, but wakefulness might be used as a provisional index, provided it is borne in mind that wakefulness and consciousness or awareness are quite different states [63,243]. Wakefulness is a state of brainstem and thalamic activity; a state of non-sleep-arousal, whereas consciousness requires cortical processing [63,243]. In order for consciousness to occur, all the incoming information from the internal and external environment must be available to all parts of the cortex at the same time [63]. Moreover, sleep is an arousable state of unconsciousness [243]. Thus, it is possible to be awake and not conscious (as in some persistent vegetative states and in conditions of blindsight [242]) and to be awake and conscious, but it is not possible to be asleep and conscious. It follows that if the fetus was shown never to be awake, it would always be unconscious. Whereas if wakefulness was evident, even transiently, it would indicate the possibility that the fetus may achieve states of consciousness, but such wakefulness would not represent unequivocal evidence for consciousness.

2.3.2. Sleep-like states in the fetus

The development of sleep-like states and behavior in the fetus has been well defined, both clinically and experimentally [17,50,110,143,168,170,186,219]. Of brief note regarding experimental models, the experimental preparation most often used to systematically study the developing fetus is that of the chronically instrumented sheep fetus. This approach permits the physiological and neurological monitoring of the fetus in utero well after surgical manipulation and without the confounding effects of anesthesia. Further, physiological responses in the fetal sheep are consistent with those observed in the human.

From mid-gestation, the fetal electroencephalogram (EEG) begins to evolve from the discontinuous patterns of tracé alternant, which is associated with the constant simultaneous expression of numerous fetal behaviors (Fig. 1), into coherent, discrete states which are suggestive of sleep and are referred to as sleep states [50,170,183]. By late gestation, as is seen postnatally, there are two well-defined sleep states, that of rapid-eye-movement (REM) or active sleep, characterized by low-voltage high-frequency EEG activity, and non-rapid-eye-movement (NREM) or quiet sleep, characterized by high-voltage low-frequency EEG activity (Fig. 2) [188]. In late gestation, these states account for 95% of fetal EEG activity, they are episodic and during each state the fetus tends to exhibit specific behaviors. Generally breathing, swallowing, licking, and eye movements, and atonia occur in REM sleep, whereas apnea, absence of eye movements, and tonic muscle activity occur in NREM sleep [50,188,219].

These observations demonstrate that sleep, or unconsciousness, is the dominant fetal state for at least 95% of the
time. The obvious and critical question is what state late-gestation fetuses are in during the 5% of the time that they are apparently not in REM or NREM sleep? Are the fetuses awake or merely in transition between the two sleep states, and how is an awake state defined? Prechtl described the categorization of state-related behavior as follows: “by the term state, one tries to describe constellations of certain functional patterns and physiological variables that may be relatively stable and that seem to repeat themselves” [182]. Postnatally, wakefulness is defined clinically by reference to opening and moving of the eyes and purposeful movements of the head; there is high muscle tone superimposed on general body movements; increased and irregular heart rate and respiration; and a low-voltage, irregular, mixed pattern of EEG with frequent movement artefacts [92,157].

2.3.3. Fetal arousal or sleep state transitions?

Using this definition of neonatal wakefulness, two “arousal” states have been suggested to occur in the human fetus [54,73,168]. The fetus is said to be actively awake (in state F4) when vigorous, continual activity, including trunk rotations, and eye movements are present and its heart rate is unstable with long accelerations [168]. Fetal “wakefulness” has also been reported in the late-gestation sheep fetus; it was characterized by REM sleep (which may contain other intermediate voltages and frequencies) coupled with the simultaneous appearance of vigorous somatic muscle activity, breathing movements, and the presence of eye activity [48,109,110,165,167,189,193,194,219]. The very brief and infrequent occurrence of this combination of behaviors typically only appears to occur as the fetus is transitioning into, or sometimes out of, NREM sleep, and this intermediate state appears to increase with gestational age [193,204,219,228] (Fig. 2). Curiously, in the fetal sheep literature at least, despite the simultaneous and almost constant occurrence of all of these behaviors earlier in gestation, at an age where brain maturation is equivalent to a 30-week-old human [148] and thus when nociceptive pathways may be intact (Fig. 1), fetuses are not referred to as being awake.

2.3.4. Evidence that the fetus remains asleep

Certainly there is no consensus on whether this constellation of simultaneously occurring “awake” behaviors truly represents a distinct state and, if so, whether it represents wakefulness; that is, purposeful directed behavior as seen after birth [110,172,188]. Indeed, surprisingly little work has been done to explore the physiology of so-called fetal wakefulness, so that the existence of this state is accepted only because it is similar to newborn activity. However, an intriguing study by Rigatto and colleagues, in which the unanesthetized sheep fetus was observed directly in utero using a Plexiglas window, showed no evidence of fetal wakefulness, defined by open eyes and coordinated movements of the head, despite some 5000 h of recording [190]. Instead, fetal behavior alternated between the two basic behavioral states of REM and NREM sleep. Moreover, they considered that the polysynaptic reflexes they observed previously, which they had speculated might reflect wakefulness, were associated with generalized tonic discharge and rotation of the body and head during transition from REM to NREM sleep [189].

Fig. 1. Example of a chart recorder tracing from a sheep fetus at 91 days of gestation (0.6 gestation, term = 147 days). This stage of maturity corresponds with the human brain at around 26–28 weeks of gestation. The trace shows changes in fetal electroencephalogram (EEG), electrooculogram (EOG), nuchal electromyogram (EMG), and tracheal pressure measurements. Note the lack of clearly defined sleep state and the near-continuous presence of eye, body, and breathing movements which typify behavior in the immature fetus. The first half of the trace is run at a higher speed to show that a variety of fetal activities frequently occur coincidentally. Bar A = 10 s, bar B = 10 min.
The considered conclusion of this review, based on the analysis outlined below, and Rigatto’s important observations, is that the fetal behavior described by others as ‘wakefulness’ in fact represents a transition phase between sleep states, a state which in the newborn is called indeterminate sleep [65,157,202]. Thus, the increasing occurrence of this state with increasing fetal age is not because the fetus is more awake, but merely reflects the increased rate of switching between sleep states with advancing gestational age [50,170,220,228].

2.4. Sleep transitions—an indeterminate state of sleep

In both the term and preterm infant, active sleep segments are interrupted by periods of indeterminate or transitional sleep [202]. Indeed, preterm infants spend a considerable time in indeterminate sleep [133]. Transitional or indeterminate sleep is a state that cannot be definitely classified as active or quiet sleep and is comprised of mixed EEG activity, increased heart rate, blood pressure, and respiration, and behavioral changes including startle-like jerking and limb thrashing [82,92,157,202]. Importantly, this transitional sleep state does not represent wakefulness; it is not, for example, drowsiness [82]. We hypothesize that so-called wakefulness in utero is likely to represent transitional sleep in view of the behaviors described, its brief nature and temporal relationship to the onset or end of NREM sleep (Fig. 2), and its similarity to post-natal sleep-arousal.

Full arousal from sleep (waking up) is a caudal–rostral brain process which originates in the ascending reticular activating system of the brainstem, spreads to the thalamus, and finally to the cortex. Importantly, however, arousal can also occur without cortical activation and waking; this is termed sleep-arousal. In the infant, sleep-arousal without waking is common [49,150,185]. The cardiorespiratory and postural changes during arousal do not represent cortical activation; rather, they are mediated by the brainstem [150,225]. In part, these periodically occurring reflexes serve cardiorespiratory homeostatic functions and also provide for several aspects of growth and development [225]. These functions are important, but critically, so too is sleep, particularly REM sleep, for normal neural maturation both pre- and postnatally [142,187]. Thus, during sleep-arousal, the caudal to rostral progression of impulses from the brainstem to the cortex is retarded by increasing inhibition which serves to decrease cortical arousal, thereby preserving the integrity of REM and NREM sleep, but at the same time allowing for essential cardiorespiratory and muscle activity [225].
Arousing stimuli, such as obstructive apnea or tactile or auditory stimulation, can produce changes in cardiorespiratory activity and behavior (e.g., limb withdrawal), even in the absence of cortical EEG changes and waking [49,149]. These observations are consistent with the general concept that during sleep there is a blunted capacity to respond to external stimuli; i.e., stimuli may reach the cortex, but sensory information is “gated”. This is due to activity-dependent depression of cortico-thalamic synapses produced by increased thalamic firing during arousal. Because cortico-thalamic cells are well coupled to inhibitory interneurons, this can result in enhanced cortical inhibition [41,83]. During indeterminate transitional sleep, cortico-thalamic responsiveness and thalamic transmission levels are the lowest of all sleep-waking stages [82]. Feedback inhibition in gating is mediated by a complex interaction of neurotransmitters [83], and the putative mechanisms of this enhanced inhibition in utero are discussed in detail below.

In the newborn where sleep predominates, gating of sensory stimuli is likely to be a key mechanism helping to keep infants predominantly asleep [116,142]. As suggested by Roffwarg’s ontogenetic hypothesis, in the fetus REM sleep may in a large part substitute for wakefulness in the development of neural network organization, providing intense sensory input not otherwise available in utero [104,192]. Studies of fetal cerebral metabolism and activity during sleep states and altered oxygenation further support this contention [187]. Moreover, reduced sensory perception, as would be experienced in utero, acts to increase cortico-thalamic gating [130]. Postnatally, it is suggested that the decline in REM sleep after birth reflects the capacity to experience an awake state and the availability of sensory input to help the brain develop [104].

Collectively, these observations strongly suggest that the fetal behavior described as wakefulness is in fact that which is typically associated with changes in sleep states and is consistent with sleep-arousal. This does not equate with being awake. Further, it is not clear what purpose wakefulness would in fact serve for the fetus given the limited duration of this state and the limited sensory input available within the “dark and muffled confines” of the in utero environment [104]. However, it is clear that while sleep-arousal does not usually terminate in waking, full arousal can and does occur in the newborn, and this is an important part of the newborns defense against acute threats to its survival during sleep. Thus, noxious and nociceptive stimuli will awaken the newborn, but can the same stimuli awaken the fetus?

2.5. Can the fetus be woken up?

If the fetus is essentially always asleep under physiological conditions, then cannot it be woken up by external nociceptive stimulation? This question has not been directly tested. In the newborn, a sufficiently threatening noxious stimulus (e.g., hypoxia or hypercapnia) will lead to full arousal, even in the preterm individual [135,150,173,225]. However, fetal responses to such stimuli are significantly different. The fetal response to hypoxia and severe asphyxia, for example, is characterized by apnea, cessation of fetal body movements, a shift to hypometabolic EEG states, and redistribution of combined ventricular output away from peripheral organs to key central organs such as the heart and brain [31,187,188,201]. Anatomically, some of these responses, such as the apneic response to hypoxia, are facilitated by unique inhibitory pathways within the fetal brain [53,79] and, as discussed below, cerebral metabolic changes are in a large part mediated by adenosine [107,120]. Similarly, while elevated CO2 tensions can elicit an increase in stimulated breathing movements if started in REM sleep, this response is inhibited when the fetus transitions into NREM sleep [191], and this is in part due to the inhibitory effect of temperature in utero [126].

These data demonstrate that stimuli which induce arousal to waking postnatally do the opposite in utero; they further suppress fetal arousal, and for that reason, promote survival of the fetus during adverse conditions. This inhibitory set of responses is a key defense strategy to conserve energy [31,187]. These responses to hypoxia also exist in very young fetuses [31,70,115], although the greater anaerobic reserves of the younger fetus appear to permit a brief period of intense movements at the onset of a severe asphyxial insult which may help the fetus extricate itself from harm [70]. However, these movements occur despite the cortical EEG switching to an isoelectric state, i.e., a state of profound suppression [30]. Thus, they are likely to be subcortically/brainstem mediated and do not reflect arousal to an awake state. This is despite the life-threatening severity of the insult and the greater quantities of cerebral energy reserves in the preterm fetus which could potentially be utilized to transition to an awake state [51,206], if such a state was important for survival. These observations are important because they demonstrate that the in utero environment is not the same as that in which the newborn lives, and this necessitates different responses from the fetus, mediated in part by unique wiring of the fetal brain. Unlike the situation postnatally, arousal costs the fetus critically limited energy resources and for little advantage.

Further evidence that fetal arousal in response to noxious stimuli is suppressed is suggested by studies of the intense and potentially “painful or distressing” stimuli used in vibroacoustic stimulation (VAS). The purpose of VAS is to evaluate the integrity of the fetal central nervous system by eliciting distinct fetal movements or fetal heart rate changes [1]. It is accompanied by variable changes in EEG activity, depending on the fetal ‘sleep state’ during stimulation [2,23,131,234]. Recent detailed analysis of the EEG, however, shows similar dynamics to those seen during spontaneous sleep state transitions rather than arousal to an awake state [204]. These data suggest the effects of VAS are mediated by actions of the brainstem structures and activation of the afferent reticular arousal system [204]. These findings are consistent with the observations that in the newborn cortical
arousal can occur in response to stimuli, without wakefulness occurring [149,225].

2.6. Can the fetus dream?

We note in passing that these observations bear on the question of whether the fetus can be said to “dream”. Suffice to say the subject of how and why we dream remains a complex and hotly debated field of research: for example, whether it has a role in consolidating conscious experiences such as learning and memory, or do we dream in order to forget [216,231]? To ask whether the fetus dreams is an equally difficult issue to address. The inherent psycho-analytical issues aside, dreams (at least postnatally) are composed in a large part by memories obtained in the awake state. Sleep itself, however, is often referred to as an “amnesiac state”: the content of sleep is poorly remembered, suggesting that the structures responsible for encoding and storing information are suppressed or absent in sleep [231]. This in turn suggests that new memories, at least declarative memories (the type of memory commonly referred to by the terms “memory” or “remembering” [231]), are not likely to be obtained during sleep.

From this perspective, due caution should be given to the hypothesis that fetuses can learn in utero and that memories based on this learning can be recalled after birth. It may be argued that neural entrainment to a repeated stimulus (such as noise) which produces a reflex response to that stimulus, as has been demonstrated recently in chimpanzee newborns after repeated exposure in utero to sound [114], is not the same as cognitive awareness or learning about a stimulus to produce declarative memory. Whether procedural or implicit memory (memory formed by unconscious acquisition and utilization of perceptual and motor skills) truly occurs in utero, or more importantly is a source for dreams, can only be speculated upon. On the whole, however, given the apparent importance for dreaming of memory obtained in the awake state, the general amnesiac aspect of sleep and that the fetus appears to be actively maintained asleep, fetal dreaming seems unlikely.

2.7. Summary of “fetal wakefulness”

In summary, this evaluation of the clinical and experimental literature shows that there is no convincing evidence to suggest that the fetus is ever awake; rather, it supports the concept that the fetus exists in a continuous sleep-like state. The evidence suggests, further, that so-called fetal wakefulness, which is said to occur between REM and NREM sleep states, is more akin to transitional or indeterminate sleep or to non-waking cortical arousal rather than an awake state. Although a state of cortical arousal can be elicited by external stimuli, it is a continuation of sleep. Indeed, evidence suggests that unlike the newborn, noxious or nociceptive stimuli do not cause cortical arousal to an awake state as a defense response; rather, the response of the fetus is typically characterized by greater inhibition. If the fetus is always asleep, it is not conscious, and therefore cannot experience nociceptive inputs as pain.

3. Suppressors of fetal behavior and cortical activity

The conclusion suggested in the section above is further strengthened by consideration of the increasing body of evidence which shows that there are several suppressors in utero which act to inhibit neural activity in the fetus to a far greater degree than is seen postnatally in the infant. The uterus plays a key role in providing the chemical and physical factors that together help to keep the fetus continuously asleep. We propose that this is achieved, among other things, through the combined neuroinhibitory actions of a powerful EEG suppressor and sleep inducing agent (adenosine), two neurosteroidal anesthetics (allopregnanolone, pregnanolone) and a potent sleep-inducing hormone (prostaglandin D2), acting together with a putative peptide inhibitor and other factors produced by the placenta, further supported by the warmth and cushioned tactile stimulation of the uterine environment.

When considering this concept, it is important to distinguish between overall neural activity and the maturation of local gating at the spinal level. For example, there is evidence from the neonatal rat that descending inhibition is markedly less than in the adult. This immaturity is suggested to reflect either delayed maturation of crucial interneurons in the dorsal horn or insufficient local levels of 5-hydroxytryptamine [67], and not overall neural activity. A proposed schema of the putative inhibitory factors can be seen in Fig. 3. In this section, we review the fetal EEG and fetal behavioral evidence for a key role for these suppressors.

3.1. Adenosine and oxygen status

Adenosine, a purinergic messenger, regulates many physiological processes in excitable tissues, especially the brain, by inhibiting metabolic activity and/or by modulating the supply of metabolic substrates via vasodilatation [61,169,180]. Adenosine, acting via its A1 receptors, inhibits many neurotransmitters, especially the excitatory glutaminergic systems, so that the net effect in nearly every region of the brain is to reduce excitability [61]. This is relevant to sleep and arousal states, as high circulating and tissue concentrations of adenosine promote sleep [169,180]. The biological half-life of adenosine is very short, well under 10 s [34,214], typically in the range of 0.6–1.5 s [159], so that the plasma and tissue concentrations of adenosine change very rapidly in response to changes in adenosine production.

In the sheep and human being, the circulating concentrations of adenosine are 2- to 4-fold higher in the fetus than the dam [20,153,200,239–241]. Although all tissues may contribute to circulating adenosine, the placenta and to a
lesser extent probably the fetal liver are the major sources [20,21,138,200,209]. Fetal plasma adenosine concentrations increase further during hypoxemia or anemia in human fetuses [196,238,239,240] and in fetal sheep [120,123] and decrease during marked hyperoxemia in fetal sheep [20,21,153,200]. The increase in local adenosine during severe hypoxia suppresses cerebral metabolic activity and helps limit neural injury [107].

As in the adult, fetal adenosine levels affect sleep state and arousal as reflected in REM-related and NREM-related EEG activity, muscular activity, and breathing movements. Infusion of adenosine reduces the incidence of rapid eye movements and fetal breathing during the first hour [120–122], but these effects are not sustained during 9-h infusions [121]. The metabolically stable adenosine analogue, 1-phenylisopropyl-adenosine, reduces the incidence of REM-related and increases the incidence of NREM-related EEG activity in a dose-dependent manner, and abolishes fetal breathing and muscular activity [221]. Conversely, infusions of theophylline, a non-specific adenosine receptor antagonist, increase fetal breathing and the incidence of REM sleep [18], and abolish the suppressive effects of adenosine infusion [121].

These data demonstrate that the higher concentrations of adenosine in fetal than in maternal plasma contribute to the predominance of sleep-like EEG activity seen before birth in the human and sheep fetus [32,44,52,93,226]. Moreover, the effects of short-term and long-term hypoxemia [37,38] and of hyperoxemia [19,95] on EEG activity, rapid eye movement, breathing, and body movements in fetal sheep seem to primarily reflect the inverse relationship between fetal oxy-
from at least 17 weeks of gestation in the human fetal CNS, skin, liver, kidneys, lungs, and small intestines [197]. Moreover, allopregnanolone and pregnanolone are present at significant concentrations in umbilical blood at birth in the human infant [36,101]. In stress situations such as hypoxia, allopregnanolone levels are actively maintained in the brain and are increased in circulating plasma [163].

### 3.3. Prostaglandin D₂

Prostaglandin D₂ (PGD₂) is a potent sleep-inducing paracrine hormone which is formed in the adult mammalian CNS from prostaglandin H₂ under the action of the enzyme prostaglandin D synthase (PGDS) [96]. Sleep induced by intracerebroventricular (icv) infusion of PGD₂ is indistinguishable from natural sleep as judged by its normal REM and NREM characteristics and by several other electrophysiological and behavioral criteria [96]. PGD₂ infused into the subarachnoid space promotes sleep by increasing the firing rate of sleep-active neurons in the ventrolateral preoptic area, adjacent to the anterior hypothalamus, and not elsewhere in the brain [96]. The mechanisms of action of PGD₂ involve paracrine stimulation of adenosine release which leads to GABAergic inhibition of wake-promoting neurons in the posterior hypothalamus [97].

PGDS is present in the cerebrospinal fluid of fetal sheep at 125 and 135 days of gestation, but not at 90 days [132]. Inhibition of PGDS action by icv infusion of SeCl₄ increases fetal behavioral activity as indicated by increased nuchal muscle, electrooculograph, and breathing activity, and icv infusion of PGD₂ reverses these SeCl₄-induced effects [132]. These observations suggest that PGD₂ has a basal role in inducing sleep in fetal sheep from at least 125 days of gestational, when discrete REM and NREM sleep states are established [52,193,194].

### 3.4. Placental peptide inhibitor and other factors

A key role for the placenta in maintaining fetal sleep states is shown by studies of “birth in utero”, in which the fetus is ventilated to achieve normal oxygenation. Under these conditions, when the umbilical cord is occluded, continuous behavioral activity and continuous breathing develop in the fetal lamb [9–11]. This paradigm mimics the effects of separation from the placenta at birth, and thus removal of its inhibitory effects. Placental adenosine and pregnanes are likely important mediators of this effect, but there is some evidence for an additional placental inhibitory factor. Infusion of a placental extract but not extracts of other fetal tissues suppresses fetal activity and respiration within 2 min [9–11]. This putative factor has not been fully characterized, but is probably a peptide with a molecular mass between 2.5 and 4.5 kDa. However, not all studies support the existence of such a placental mediator [125].

Other inhibitors are also likely to contribute, for example, neuropeptide Y (NPY), somatostatin, enkephalins, and corticotropin releasing hormone (CRH) [100,213]. Postnatally, NPY is known to have sleep promoting and sedative actions and to reduce pain [161,235]. The levels of NPY are relatively high in the fetal brain and decline after birth [100], and during fetal life are known to play a role in fetal responses to asphyxia [199]. Other peptides, such as substance P, which is a primary sensory transmitter mediating pain sensations via the thin C-fibers, markedly increase expression and function after birth [33,184]. While CRH is well known to be produced during stress and mediates many of its effects, it is less well appreciated that it also has analgesic effects at all levels of the neuraxis [129], which may augment the overall protective role of this system during stress. Likewise, placental CRH in human beings is speculated to play a role in mediating the rapid attenuation of fetal responses to repeated stimuli [198], and needling procedures appear to stimulate CRH release from placental not fetal sources [75]. In the late-gestation sheep fetus CRH is known to play a critical role in modulating the episodic nature of fetal behavior [29]. Also, endogenous activity in the kappa opioid system may be functional in modulating responses to the sensory environment; in particular, it may regulate the rapid fetal habituation to sensory input [213].

Finally, growth hormone (GH) levels are high in fetal life, but unlike the situation postnatally, GH does not significantly contribute to fetal growth [108]. There is a positive relationship, however, between GH and sleep [227], and sleep-related GH secretion is probably caused by an increased production of hypothalamic growth-hormone-releasing hormone acting on the pituitary [227]. Speculatively, the high levels of GH seen during fetal life may reflect an important role for growth hormone in keeping the fetus asleep. A similar role may be postulated for ghrelin, an endogenous ligand for the growth hormone secretagogue receptor, which may be secreted both by the fetus and the placenta [117,229].

### 3.5. Thermal status—warmth

Sleep-wakefulness and body temperature are known to influence each other. Postnatally, we know that warmth can make us drowsy; sleep is effectively promoted by a hot bath, for example [105]. There is evidence that fetal thermal status affects the level of behavioral activity in utero, i.e., that warmth suppresses it. The work by Kuipers and colleagues suggests that temperature modulates the state-related fetal respiratory response to elevated CO₂ tensions [126]. Human, sheep, and other fetuses are slightly hyperthermic in relation to their mothers because the heat they generate can only be dissipated down a thermal gradient across the placenta [3,80,181]. Cooling mature well-oxygenated fetal sheep in utero, such that their cutaneous thermoreceptors are exposed to cold via cooling coils in amniotic fluid, elicits behavioral activity, shivering, and increased respiration, and
rewarming such fetuses reverses these effects [80,88–90,181]. In utero cooling of the fetal skin using amniotic fluid cooling coils apparently induces EEG changes indicative of arousal [203]. Immersing aroused, physically active, and conscious newborn lambs in water at maternal body temperature induces a sleep-like, non-aroused, unconscious state, and cooling the water restores their prior aroused, conscious, and physically active state [62].

The translation of thermal information from the skin to induction of sleep may occur via temperature-sensitive neurons within the hypothalamus [147,154]. Various regions of the hypothalamus (e.g., the hypothalamic preoptic area and prefrontal areas of the lateral hypothalamus) are involved in both the regulation of sleep-wakefulness and body temperature [147,154]. Activation of sleep-related warm sensitive neurons and the deactivation of wake-related cold-sensitive neurons appear to play a role in the onset and regulation of NREM sleep [6]. Further, warmth inhibits neuronal discharge in arousal-related brain structures, including regions which promote wakefulness such as the dorsal raphe nucleus [6,91,147]. These responses may be mediated by multiple inhibitors such as adenosine, GABA, prostaglandins, and growth hormone [7,98,145,218,244]. In this way, the constant moderate ambient warmth experienced by the fetus may facilitate a constant state of sleep.

3.6. Tactile stimulation

Tactile sensory input is minimized in utero by amniotic fluid which buffers the embryo/fetus against mechanical stimulation and presumably also reduces sensations associated with gravity. In contrast, intense tactile stimulation during labor and after delivery is considered to contribute to the usually rapid onset of behavioral activity and consciousness in newborn human infants [127,128] and other newborn animals, including lambs [151].

3.7. Summary of endogenous inhibition

This analysis suggests that the suppression of fetal wakefulness-related CNS activity and behavior described in the first part of this review is achieved to a significant extent by the combined effects of multiple neurotransmitters, peptides, and endocrine factors coupled with warmth, buoyancy, and cushioned tactile stimulation (Fig. 3). During stress situations, CNS suppressor effects are often significantly increased, and this leads to an even deeper inhibition of the fetus, providing, we argue, a greater degree of “endogenous anesthesia”. Clearly much more work is required to dissect the relative importance of each of these systems and to understand fully how the systems interact. Nevertheless, the existing data strongly suggest that the fetal response to noxious and nociceptive stimuli is significantly different from that seen after birth and is in large part due to its unique environment, with the placenta playing a key role.

4. Long-term sequelae

Here we consider one final issue: whether nociceptive inputs may have deleterious consequences even if the “endogenously anesthetized” fetus does not consciously perceive pain at the time of stimulation. Can exposure to noxious stimuli initiate a cascade of events that sensitize the nervous system [84], or can repeated pain exposure in preterm infants contribute to attention, learning, and behavior problems later in life [35,87,237]? It is critical to appreciate that not only is most of the available information based on studies in preterm infants, and not the fetus, but also there are no direct studies which have robustly tested these speculations, even in the preterm newborn, and thus no empirical data to demonstrate a causal relationship.

It is striking that where “stress” or treatment with steroids has been found to affect the fetus [45,46,55,86,162], the “stress” has been chronic, often extending over a third or more of gestation, whereas brief treatment seems to affect development only when given very early in gestation [58]. Moreover, the fetus is likely to be naturally exposed to elevations in “stress” hormones, for example, during periods of hypoxia or other stresses [77]. Such exposure may occur frequently throughout development, particularly during early to mid-gestation [27,70], and circulating cortisol concentrations increase markedly in late-gestation, staying elevated until several weeks after birth [144]. Thus, it is likely that a brief period of fetal hypothalamic–pituitary–adrenal (HPA) activation in mid- to late gestation in response to short duration surgery-induced nociceptor input would have limited importance.

In summary, considerably more work is required to evaluate the long-term consequences of nociceptor activation in the fetus and indeed the premature newborn. Until then, we, like Grunau, advise against overinterpreting correlational studies and limited data [87].

5. Summary and conclusions

We have considered whether the fetus, once its nociceptive pathways are complete, can feel pain in utero in a psychological manner akin to adult pain experience, and whether regardless of this the physiological responses to nociceptive input may lead to altered behavior later in life. We conclude that there is currently no strong evidence to suggest that the fetus is ever awake, even transiently; rather, it is actively kept asleep (and unconscious) by a variety of endogenous inhibitory factors. Thus, despite the presence of intact nociceptive pathways from around mid-gestation, the critical aspect of cortical awareness in the process of pain perception is missing. Furthermore, there is currently no direct evidence to suggest that subcortical effects of nociceptor input in the fetus can alone alter neural development and cause long-term adverse outcomes. Rather, the inference that this may occur is derived from studies of
chronic maternal “stress” or administration of synthetic glucocorticoids, or speculation about the potential vulnerability of immature neurons.

It is of concern that such hypotheses about the impact of nociceptive stimuli are readily accepted as fact and that this perception in turn instructs our clinical understanding about the management of presumed pain in utero. The influence of the postnatal environment and of cognition and learning on modulating these effects is clearly quite considerable; an influence that has not been considered in most studies and reviews. Furthermore, key questions have typically not been addressed when considering the potential impact of presumed pain in utero. For example, what is the inter-relationship between the relatively diffuse immature pain pathways [136], and the overall inhibition from endogenously-produced analgesic neurotransmitters during noxious stimuli? Another example is that the stress response to noxious stimuli includes release of CRH which has analgesic effects in its own right in the fetus but not postnatally [39,160]. The fetal response to nociceptive input is clearly more complex than we currently appreciate, and as highlighted above appears to be ‘engineered’ to further anaesthetize the fetus during exposure to noxious stimuli. It may be strongly argued that these factors interact to significantly ameliorate, if not eliminate, the deleterious impact of nociceptive input in the fetus compared to the effects postnatally.

Surprisingly, despite advocacy for analgesic use during fetal surgery, the impact of analgesics and anesthetics on brain and other organ development has not been extensively studied. Yet there is good evidence to show that such agents affect the fetus quite differently and may cause fetal compromise. In fetal sheep, morphine, for example, is not a cardiorespiratory depressant; rather, it induces continuous fetal breathing movements (FBMs) which increase metabolic demand [28,42]; it also stimulates the fetal HPA axis [223] and alters fetal glucose handling [222]. In utero, opiates do not necessarily have analgesic properties and may increase fetal compromise during stress situations [124,141,212]. Importantly, there is now evidence to show that exposure of the fetus to opiates may cause significant neuronal and white matter cell loss [81,106,207,217].

Non-narcotic analgesic agents have also not been evaluated extensively [177], but may have similar consequences. For example, indomethacin also significantly increases FBMs [4] and may alter the fetal responses to hypoxia [103]. In newborn immature rats, at least indomethacin administration is associated with subsequent impaired neural development [26]. For all agents, given the diffuse nature of pain receptivity and the limited fetal capacity to metabolize many agents, it is difficult to judge dosage and the required duration of treatment, and it is difficult to find a safe route which can be used repeatedly or chronically.

In the adult, some experimental studies have indicated that pre-emptive analgesia which blocks the intense afferent barrage of impulses received by the subcortex and brainstem during surgery significantly modifies post-surgical pain [152]. These findings are, however, not supported by a recent major meta-analysis of clinical trials, mainly in adults, which found no evidence that pre-operative analgesia can substantially reduce post-injury pain hypersensitivity [158]. Although there are few large studies in preterm newborns, a randomized controlled trial of pre-emptive analgesia with morphine was associated with worse outcomes [14], no appreciable change in pain scores [208], and no reduction in adverse neurological outcome [14,208]. Indeed, there is some evidence that prolonged exposure to analgesic drugs may detrimentally alter neuronal and synaptic organization permanently [12].

What we hope is apparent from this review is that this important issue is clouded by inappropriate extrapolation from the postnatal state to the fetal condition. The assumption that the newborn can act as a surrogate for the fetus is wrong, for it denies that the environment in which the fetus lives is unique, and that the fetus itself responds quite differently to many challenges. Greater scientific rigor is required to directly address the concept of fetal awareness in utero as part of understanding presumed fetal pain perception, and of the potential impact of nociceptor input on neural development and subsequent behavior. It is not enough to say, “If in doubt treat”, for treatment may produce greater adverse outcomes than the effects of relatively transient nociceptor input. Certainly, it is the conclusion of this review that in order to better understand the nature of presumed fetal pain, we must first evaluate the fetus and its unique in utero environment from the viewpoint of the fetus; not as a newborn not yet born or as an adult who is simply waiting to grow up.

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