Responsiveness to painful stimuli in anaesthetised newborn and young animals of varying neurological maturity (wallaby joeys, rat pups and lambs)

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Abstract

The patterns of neurological development, as indicated by electroencephalographic (EEG) activity, are similar in different species. EEG activity is absent (isoelectric) initially. It then shows intermittent spikes followed by longer more sustained epochs that are separated by isoelectric periods. Thereafter, a continuous undifferentiated EEG appears and this finally differentiates into alternating rapid-eye-movement (REM) and non-REM sleep-like patterns, with a later addition of characteristics indicating conscious awareness. The timing of these developmental changes evidently depends on the neurological maturity of the young at birth. In wallaby joeys (extremely immature), the EEG evidently remains isoelectric until about 100-120 days of in-pouch age and becomes continuous by about 150-160 days, with behavioural signs of conscious awareness apparent by about 160-180 days. In rat pups (immature), the EEG is isoelectric, intermittent or continuous but undifferentiated at birth with REM/non-REM differentiation occurring between postnatal days 12-18, and conscious awareness appearing no earlier than this. In lambs (mature), REM/non-REM differentiation occurs before birth (at 0.8 pregnancy) with conscious awareness appearing only minutes after birth. Pain-specific EEG responses of anaesthetised young of these three species apparently depend on neurological maturity and, in lambs, on proximity to birth. These observations have some novel implications for pain management during studies of newborn animals.

Keywords: neonatal maturity, pain management, refinement

Introduction

The prevention or alleviation of negative mental states, such as pain, is important for good animal welfare, so that the provision of anaesthesia and/or analgesia is a required refinement when animals are subjected to invasive and significantly painful procedures.

Newborn and young animals of various species have been used in developmental research for decades. Such research has advanced our understanding of normal and disordered function and has led to vast improvements in the treatment of newborn human infants as well as improved health and welfare in newborn farm and laboratory animals (Mellor and Gregory, 2003). As with adult animals, the aversiveness of certain experimental procedures may have been judged using a variety of measures, including withdrawal of body parts, vocalisations, heart and breathing rate changes as well as changes in stress-related hormones such as adrenaline and cortisol (Lee et al., 2005; Mellor et al., 2005). However, the presence of these responses does not necessarily indicate conscious perception of pain, i.e. pain experience. Physical withdrawal and vocal reflexes as well as activation of body stress responses can be elicited by stimulation of lower brain centres via pain pathways (Lee et al., 2005; Mellor et al., 2005; Mellor and Diesch, 2007) and hence do not require the involvement of the cerebral cortex and conscious awareness. While in the healthy conscious adult these indices give us a reasonably good indication of the status of the animal with regard to pain experience, this may not be so in immature newborn or young animals (Ellingson and Rose, 1970) whose nervous systems are still developing the capacity to allow pain to be consciously experienced.

For any animal to experience pain and to suffer from it, two prerequisites have to be met (Mellor and Diesch, 2006). First, the animal has to be sentient. This means that the animal's nervous system has to
be sufficiently developed to relay sensory inputs (e.g.,
electrical impulses) from the periphery to the higher
centres of the brain (i.e., cerebral cortex), where
such impulses can then be interpreted. Second, the
animal has to be conscious, as unconscious animals
cannot experience pain, for instance during general
anaesthesia.

The developmental stage at which animals are
capable of experiencing pain therefore depends on
when they become sentient and consciously aware, i.e.
when they are able to consciously perceive sensations.
While research over the past few decades has vastly
improved our knowledge about the neurological
development in a variety of species, we do not yet
know clearly when conscious perception emerges in
most species.

Neurological development and its importance for
conscious awareness

The overall pattern of neurological development is
similar in different species of mammals, and the
timing of birth during the course of this development
determines whether animals are born relatively
mature (e.g., sheep, cattle, horses and other ungulates),
moderately immature (e.g., mice, rats, cats and dogs)
or extremely immature (marsupials). This can be
illustrated by looking at the development of brain
electrical activity, the electroencephalogram (EEG).
Initially, the EEG is absent, it is isoelectric, but
intermittent spikes develop and then longer more
sustained epochs of EEG activity, which are also
separated by isoelectric periods, become apparent.
Subsequently, continuous EEG activity appears.
This then differentiates into alternating rapid-eye-
movement (REM) and non-REM sleep-like patterns,
with later addition of characteristics indicating
conscious awareness. However, the timing of these
changes in relation to birth differs between species. In
those born extremely or moderately immature REM-
non-REM differentiation occurs some time after birth,
whereas in those that are neurologically mature at
birth such differentiation occurs during late pregnancy
(Ellingson and Rose, 1970; Mellor et al., 2005).

It has been suggested that the neural functions
required for REM and non-REM sleep are linked
to brain mechanisms that also support conscious
awareness (Evans, 2003). Accordingly, REM-
non-REM differentiation may be used to indicate
the earliest stage at which brain mechanisms may
have matured sufficiently to support conscious
perception in those species where this occurs after
birth. However, this is not apparently the case in
those species where REM-non-REM differentiation
occurs before birth because of the operation in utero
of a range of potent neuroinhibitory mechanisms
that maintain states of unconsciousness in the fetus
throughout the last half of pregnancy (Mellor et
al., 2005). In these species, therefore, conscious
perception occurs only after birth (Mellor et al., 2005;
Mellor and Diesch, 2006).

Overall, therefore, we would not expect conscious
perception to become apparent before the age at
which REM-non-REM differentiation occurs,
as underlying neurological structures and their
interconnections would not be sufficiently mature
and functionally competent to support conscious
perception. However, even if underlying neurological
structures are mature and functional, the first
appearance of conscious perception may be delayed
if environmental factors are present to suppress it, as
seen in the neurologically mature late-gestation fetus.

Responsiveness to noxious stimulation in
anaesthetised newborn and young animals

Changes in EEG parameters have been reported
to reflect changes in cerebral activity associated
with pain perception (Bromm, 1984, Chen et al.,
1989). Previous studies show a similarity in the
EEG response to potentially painful stimulation in
conscious and anaesthetised animals (Ong et al, 1997;
Murrell et al, 2003) and in conscious humans (Chen
et al, 1989). We have therefore used the EEG as a
means of studying the ability of newborn and young
animals to experience pain.

Cerebrocortical (EEG) responses to potentially
painful stimulation were studied in lightly
anaesthetised tammar wallaby joeys (marsupial), rat
pups and lambs. The use of general anaesthesia means
that this is a humane approach for investigating brain
processing of impulses in pain pathways (Murrell and
Johnson, 2006; Johnson et al., same proceedings).
Although the animals were therefore unconscious,
inferences can be made about whether the cerebral
cortex is mature enough to respond to potentially
painful stimuli at the ages investigated, and thus,
whether the animals would be capable of consciously
perceiving pain had they not been anaesthetised.

Extremely immature at birth: Wallaby joeys

Marsupial young are extremely immature at
birth and most neurological development occurs
postnatally while they are in the mother's pouch
(Tyndale-Biscoe and Janssens, 1988). The joey of the
tammar wallaby (Macropus eugenii) for example,
is born after a gestation of only about 28 days. It
emerges from the female reproductive tract and pulls
itself into the pouch where it attaches to a teat. At
birth the developing cerebral cortex of the tammar
wallaby consists of two layers of cells and apparently
resembles that seen in early human (40 days) and
sheep (26 days) embryos (Reynolds et al, 1985). The
joey's eyes open at around 140 days of in-pouch age,
they can stand unaided by 160 days, they begin to
"look out of the pouch by about 180 days, and their
These results, in addition to the above information on parameters investigated in response to clamping, showed a marked response in all aged 21-22 days (after REM-non-REM differentiation investigated when their tail was clamped, while those showed a moderate change in some EEG parameters 12-14 days (during REM-non-REM differentiation) between 12-14 and 21-22 day pups. Pups aged in EEG power in all frequencies investigated (1-30Hz) response to clamping. Overall, there was an increase in EEG power in all frequencies investigated (1-30Hz) from 140-180 to 187-260 days, indicating maturation of cerebral functioning. Also, the cerebrocortical response to a toe clamp was relatively smaller in joeys aged 140-180 days compared to those aged 187-260 days.

These results, in addition to the information on joey age in their responsiveness to potentially painful stimulation. Second, although pain experience may be due to the slow postnatal waning of the secretion of hormonal factors with anaesthetic, sedative and analgesic properties that are synthesised before birth in lambs, it is not likely to be an "on-off" phenomenon. Rather, behavioural observations suggest that its onset is gradual, such that its initial manifestations take minutes to appear, and other features are not apparent for several hours or even days. This is supported by our recent study showing significant maturation of the unanaesthetised lamb's EEG (i.e. an increase in EEG power) between 1-4 hours and 1-2 days after birth. Moreover, anaesthetised lambs at 1-2 days after birth show reduced EEG responses to castration compared to lambs at one week of age and older (Johnson et al., submitted). We have speculated that this may in part be due to the slow postnatal waning of the secretion of hormonal factors with anaesthetic, sedative and analgesic properties that are synthesised before birth by the fetal brain (Mellor and Diesch, 2006; Mellor et al., submitted).

Onset of conscious perception

From the results of the above studies conclusions relating to the onset of conscious perception can be drawn. First, the onset of conscious perception does not seem to follow an "on-off" phenomenon; rather it appears to develop gradually, even in those species that are neurologically mature at birth. This view is supported by the increases in overall EEG power with age observed in all three species reported on here and the concomitant increases with postnatal age in their responsiveness to potentially painful stimulation. Second, although pain experience may be qualitatively different in younger animals than...
in older and/or more mature animals, on the basis of the precautionary principle, when significantly invasive procedures are planned, pain relief should be provided from those postnatal ages when pain may first be perceived – i.e. from about 120 days in the tammar wallaby joey, about 10 days in the rat pup and from birth in the lamb.

Implications for pain management

It could be argued that analgesia and/or anaesthesia are not necessary in young animals that have not attained the capacity for conscious perception, as they will not be able to experience pain. However, there is merit in being cautious. Even when such newborn and young animals are not capable of experiencing pain, invasive procedures will nevertheless stimulate pain receptors and will elicit impulse barrages in those nerve fibres that have developed by the time the procedures are undertaken. This in turn will lead to physiological and behavioural responses, including the withdrawal of the stimulated body parts, stress hormone release and so forth (see above), all of which are mediated by lower brain centres. Such responses can negatively affect the procedure (i.e. movement during surgery) or have the potential to 'contaminate' the physiological data to be collected. Additionally, the potential for long-term changes due to spinal plasticity should be taken into consideration where animals will be maintained following exposure to potentially noxious procedures.

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