Rapport

Fosterets evne til å oppleve ubehag, smerte og stress

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Innholdsfortegnelse

Risk assessment on mammalian foetal pain, and effects of prenatal stress in offspring from mammals, birds and fish

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Background

In domesticated farm animals concern has been raised whether stress or pain sensation is possible during foetal life in relation to maternal stress, such as handling and transportation. There is also a debate going on in the human medical society whether the foetus feels pain during abortion or during intrauterine surgery. Concerns have been raised because of the observation that the foetus is able to mount stress responses to painful stimulation and maternal stress. Furthermore, studies on animals and humans have demonstrated that harmful stimulation during the foetal and neonatal period may produce permanent developmental changes. The risk of the foetus of feeling pain, and that painful/stressful exposures may cause irreversible effects on normal development, has implication for animal and human welfare and health.

Task

In 2004 the Norwegian Food Safety Authority asked the Scientific Committee for Food Safety to prepare a scientific evaluation on whether the mammalian foetus is able to sense or feel discomfort, pain and stress, and effects of prenatal stress on behaviour, physiology and morphology of offspring from mammals, birds and fish.

Results

In response, two assessments have been conducted. The first reviews the literature on foetal pain, (Scientific evaluation on whether the mammalian foetus is able to sense or feel discomfort, pain and stress, Lyche, J.L.), and the second (Effects of prenatal stress on behaviour, physiology and morphology of offspring from mammals, birds and fish, Janczak, A.M., Eriksen, M.S. and Braastad, B.O.) reviews the literature on effects on prenatal stress in mammals, birds and fish. In this report the two assessments are presented as individual manuscripts. The report is a co-operation between The Norwegian School of Veterinary Science (NVH), Oslo, Norway and The Norwegian University of Life Sciences (UMB), Ås, Norway.

The following conclusions were presented in this report

Conclusions

Pain pathways and cortical and sub cortical centres involved in pain perception are well developed late in gestation. Moreover, the neurochemical systems associated with pain transmission are intact and functional. Theoretically, in humans, pain perception becomes possible at about the beginning of the last trimester. This may also be valid for other mammalian species, such as some domesticated farm and laboratory animals. Even though the anatomic and physiologic systems for pain sensation and perception are fully developed in late gestation, the foetus may not be able to feel pain because it appears to be unaware until after birth. A strong evidence for this hypothesis is that normal levels of O_2 in the circulation of the foetal lamb are below the concentrations required to support awareness in neonates and adults. Further research is needed to elucidate whether this is the case in all mammals. The use of pain relief may be considered as a precautionary principle if major invasive techniques are to be used on foetuses in late gestation.

Stress during pregnancy has for a long time been known to affect embryonic survival and mortality, and is therefore important for the reproduction of farm animals. In addition, evidence from animal and human studies indicates that prenatal stress produce permanent developmental changes such as impairment of the stress-coping ability, disruption of behaviour in conflict-inducing situations, and on reproductive success in the first, and sometimes also in the second, generation.. Those effects may be related to increased or prolonged activity in the HPA axis produced by impaired negative feedback of glucocorticoids in the hippocampus, although several other neuroendocrine pathways may be involved.

Scientific evaluation on whether the mammalian foetus is able to sense or feel discomfort, pain and stress

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Introduction

During the last few years increasing attention, both scientifically and emotionally, has been raised in the human medical literature, as to whether a foetus is able to feel pain. Pain sensation in the human foetus is a serious and difficult issue related to late abortion, increasing numbers of intrauterine operations, and the treatment of premature children.

Previous studies of neurological development concluded that foetal and neonatal responses to painful stimuli do not include the brain cortex and that perception or localization of pain was not present. Moreover, because foetus and neonates may not have memories of painful experiences, they were not considered capable of interpreting pain in a manner similar to that of adults [1]. These traditional views have led to a widespread belief in the medical community that the foetus or neonate may not be capable of perceiving pain. One result of this pervasive view was that that foetuses and neonates were frequently not given analgesics or anaesthetics during invasive procedures, including surgery.

In farm animals concern has been raised whether stress or pain sensation is possible during foetal life in relation to maternal stress, such as handling and transportation. Furthermore, a significant number of pregnant ruminants are slaughtered annually and their foetuses die in utero from hypoxia and hypercapnia, or in some instances from foetal blood (serum) collection. This raises the question whether or not the foetuses suffer before they die. The capacity of the foetus to sense, or to be aware of, pain also has implication on human management in situations where foetotomy is necessary, and during foetal surgery.

The essential question is whether a foetus feels pain? In order to answer this question we need definitions of ‘feeling’ and ‘pain’. The International Association for the study of pain defines pain as “an unpleasant sensory and emotional experience associated with actual and potential tissue damage” and emphasises the importance of experience related to previous injury. Feeling is defined as ‘to experience a sensation’. These definitions mean that the central nervous system reaches a certain level of functioning, as well as having an awareness or consciousness of prior experience, before pain can be understood.

The risk for the foetus of feeling pain has implication for animal and human welfare and ethics. Moreover, there is increasing evidence that early painful or stressful events can sensitise an individual to later pain. Animal studies indicate that exposure to stress or pain during development can have long-term effects on hippocampus development and stress behaviour [2;3].

There is no objective measurement of pain. It is a subjective experience, but the foetus, whether it is human or animal, is unable to tell us if it feels pain. Pain consists of two components, the sensation of the stimulus (nociception), and the emotional reaction, that is the unpleasant feeling due to a noxious stimulus. These two components occur in the brain in two, both anatomically and physiologically distinct systems [4;5] Thus, the difference between the terms nociception and pain is that the first refers to the anatomical and physiological system of noxious sensation, and the second involves the perception of an aversive stimulus that requires the individual to be conscious or aware of the stimulus (i).

Sensing pain requires a developed neural pain system, which includes peripheral pain receptors, afferent neural pathway to spinal cord, ascending spinal tracts to the thalamus, and from the thalamus to the cortex [6]. Pain impulses are also processed in a number of other subcortical structures such as the hypothalamus-pituitary system, amygdala, basal ganglia, and the brain stem [4;5;7]. These brain areas account for the subconscious processing of pain and for pain-trigged autonomic and hormonal reflexes. These components of pain processing do not require cortical level activity, and they may therefore be regarded to occur subconsciously [8].

The issue of foetal pain appears to depend upon two issues. Firstly, when and how the key parts of the nervous system become capable of responding to a tissue damaging stimulation. This issue requires information on the development of the sensory pathways involved in the

sensory component of pain, such as when various pathways connect, when impulses arrive at various brain centres, and what and when bodily reactions (e.g. reflexes and stress hormone release) can be evoked.

The main problem comes with the second issue, which is the development of consciousness or awareness of pain. Consciousness or self-awareness cannot be simply localised within the brain, but is commonly accepted to require the cortex. In humans, the connections from the thalamus to the cortex begin to form from about mid-gestation and continue to mature along with other cortical connections well into childhood and adolescence [9]. At present, it is not possible to define a brain area that requires maturation before a foetus is able to feel pain. As will be discussed later in the text, the ability for a foetus to feel pain may not only depend on the degree of brain development, but may also be related to other parameters such as foetal O_2/CO_2 tension and hormone levels [10].

Measurement of pain

Measurements of pain with cooperative subjects are based on subjective scales of pain intensity, and obviously such methods are not applicable on animals, foetuses and human neonates. Therefore, a number of indirect methods have been developed in order to assess possible pain. These methods are based on behaviour (motor movement pattern), autonomic parameters (heart rate, blood pressure) and endocrine responses. They are still being developed because no one is yet able to accurately assess pain in foetuses. For example the key question is yet to be answered, do the present pain treatments (analgesic procedures) only suppress the responses to pain rather than killing the pain itself [11]?

Development of nociception

Knowledge on the ontogeny of normal growth of the nervous system is important in order to predict at which stage during foetal life a foetus may bee capable of feeling pain. A comparison of human development with that of the rat has shown that although the speed is more rapid, the sequence of development in the rat pup is very similar to the human foetus [5]. The literature on the development of the somatosensory pain system in domestic animals is scarce. However, similar to the human development, ovine, bovine, equine and porcine foetal development is precocial, and consequently the human time frame and sequence for nociceptive development may also be representative for domestic animals, although there may

be differences between species. For example, the velocity curve of pig brain growth corresponds closely with the velocity curve of the human brain [12;13].

The neuroanatomical pathways for touch and pain sensation are amongst the first to develop, suggesting pain as an important signal in early life. [1]. In the human foetus, dorsal horn cells in the spinal cord have formed synapses with developing sensory neurons by gestation week 6. These sensory neurons grow peripherally to reach the skin. Nociceptors appear around the mouth by week 7 and by week 20 those are present all over the body. By term, the foetus has the same density of nociceptive nerve endings in the skin as an adult. The central connection in the spinal cord occurs in parallel with the development of peripheral afferents. Development of various types of cells in the dorsal horn, along with their laminar arrangement, and synaptic interconnections and specific neurotransmitter vesicles begins before gestation week 13 and is completed by week 30 [5]. A functional spinal reflex circuit develops almost simultaneously with the formation of synapses between the peripheral afferents and the spinal cord [5].

Far less is known about the development of the higher part of the pain pathways. Connection between the spinal cord and the thalamus are established by gestational week 20 and myelination is completed by week 29. The timing of the connection between thalamus and cortex is of crucial importance for cortical perception, since most of the sensory pathways to the neocortex have synapses in the thalamus. The connection between thalamus and cortex begins to grow at weeks 24-26, suggesting that pain impulses are able to reach cerebral cortex for the first time during that time period [5]. However, arrival of sensory impulses at the cortical level cannot be detected before week 29 and some authors claim that the cerebral cortex starts to operate from that time onwards [8;14]. The facts that electroencephalographic activity appears for the first time at week 20, and becomes synchronic at week 26, suggest an earlier pain perception [4]. The actual time point is far from certain and has been challenged in both directions, with some arguing for pain perception at an earlier stage [15;16] and others, that pain perception does not occur until after birth [17].

Maturation of pain modulating, descending pathways in the spinal cord, which are crucial for proper pain reactions, occurs relatively late in the development. The strong reflexes to pain stimuli observed in foetuses and neonates are probably due to this immaturity of the modulatory systems, suggesting that there is less control of the entry of the peripheral stimuli into the central nervous system [8].

The pain response

A painful stimulus induces motor movements such as withdrawal reflexes and body movements, which are often regarded as positive signs of pain perception in the foetus. Foetal movements begin early in pregnancy [18]. Initially they are uncoordinated and involve local movements of the limb, trunk or neck. As the foetus mature, the movements become more coordinated and exhibit alternating periods of activity and inactivity [18]. These changes reflect the developmental progress from rudimentary precursors of nerve tracts and brain structures to sophisticated and operationally effective structures that are present at birth. In the human foetus, head tilting in response to perioral touch is observed at week 7, hands respond to touch at week 10, and at week 14 the lower limbs begin to participate in reflex movements [5;8;19]. It is important to emphasise that these reactions are completely reflexive, and it is irrelevant to speculate on perception of pain at this early stage [5;8;20]. For example, similar reflexes occur in anencephalic foetuses from week 16 until week 35, even when only parts of the spinal cord are intact [21].

Due to the immaturity of the pain-modulating system, reflex inhibition is low and hyper-reflexes are observed during the foetal period. For example, in rat neonates a prick on the hind foot may result in a whole body movement [22]. A simple interpretation on these exaggerated responses is that the foetus is highly sensitive to painful stimuli. However, there is no correlation between intensity of the pain stimulus and the strength of the reflex, and the hyper-reflexes are probably an indication of the immaturity of the inhibitory systems rather than a consistent indicator of pain.

In contrast to other motor reflexes facial expressions has been claimed to represent a specific symptom of pain. Facial expressions observed in adults during a period of pain have also been observed in premature babies as early as week 26, suggesting that babies are able to feel pain at week 26. However, facial reflexes have been shown to be triggered by various somatic stimuli at very early stages of development and are most likely coordinated by cortex independent sub cortical systems[23]suggesting that facial expressions observed in early foetal life are pure reflexes.

Autonomic and endocrine reflexes

Hormonal and metabolic responses to painful stimuli have been demonstrated during foetal development. Cardiovascular responses comprise the main physiological component of a stress response. For example, both in human and sheep foetuses it has been demonstrated that painful procedures have produced haemodynamic changes and this have lead to the conclusion that increases in heart rate and respiratory rate indicate the feeling of pain [24;25]. However, in the human foetus, circulatory stress responses to physical insults are observed before the connection between thalamus and cortex is established [25]. Moreover, in the adult rat, formalin injection into the paw produces an increase in the heart rate (first phase) lasting about 10 min followed by a period of quiescence, and then a resumption about 30 minutes after the initial injection [second phase; [26]]. In contrast, in the rat pup, which is a altricial species and early postnatal development represent prenatal development in humans, ruminants, pigs and horses, this biphasic tachycardic response does not appear before around week 3 of age, indicating a different response to chronic pain during development. Taken together, those results suggest that the foetal tachycardic response may be reflexive during development and not a consistent parameter of foetal pain.

Foetal pain has long been studied by demonstrating neuroendocrinological reactions to painful stimuli [27]. However, interpretation of those reflexes is complicated because they are relative unspecific indicators of subjective pain. For example, evaluation of circulating stress hormone levels and behaviour responses associated with osteoarthritis in dogs revealed that there was too much individual variation in the levels of stress hormones to be of use in pain assessment [28]. One study demonstrated an elevation of cortisol and β-endorphin levels in plasma in 23-week-old human foetuses following painful stimulation [29], and this study gave rise to a widespread speculation that the foetus was able to feel pain already at week 23 of age regardless of an established connection between thalamus and cortex. Based on the current knowledge on the development of the neuroendocrine system, a more reasonable explanation may be that the stimulation was able to activate the hypothalamus-pituitary axis (HPA) to produce a hormonal reflex independently of the cortex.

Prenatal pain and prolonged responses

Knowledge on the development of pain pathways provides a theoretical time frame for the ability of sensing painful stimuli during foetal life. A foetus responds to painful stimuli by various responses at relatively early stages of gestation. However, even though the foetus is capable to mount such protective reflexes, there is no evidence to support the claim that the

foetus is able to feel pain at early stages. Theoretically, the human foetus becomes capable of feeling pain following the establishment of the connection between thalamus and cortex during weeks 24-26 of gestation. However, painful stimuli are able to activate sub cortical systems of the brain, such as the brain stem and the hypothalamus-limbic systems prior to the establishment of the hypothalamus-cortical connection. Even though the foetus is not able to feel pain at early stages, there is increasing evidence that early exposure to noxious stimuli may produce permanent developmental changes. In other words, painful stimuli may not need to penetrate consciousness in order to irreversibly alter central nervous development. Furthermore, painful stimuli in late gestation have also been shown to cause irreversible effects on later development [20;30-32].

This phenomenon may be explained by early 'programming' of the brain. The term 'programming' has been used to describe the process whereby a stimulus or input during a sensitive period of development has permanent effects on the structure, physiology, and metabolism of the body, including the brain [33]. A sensitive period is defined as a specific time period during central nervous development in which the effects of experience can alter neuronal connectivity. Even small variations in foetal physiological environment in specific periods of time may mediate the effects on later development. For example, small variation in endogenous steroid hormones produced by the gonads, the thyroid and the adrenal glands have been shown to exert programming effects on the developing brain. [2;34;35].

Behavioural arousal and awareness in foetal life

As discussed earlier, the ability to feel pain appears to depend on the two issues: When and how the key parts of the nervous system become capable of responding to a painful stimulus, and whether the foetus is conscious or aware of the pain. The chronically instrumented sheep preparation has been widely used for biomedical and veterinary studies on perinatal physiology and pathophysiology, and as a consequence, this has propagated knowledge on ovine prenatal development [36-40]. By catheterizing foetal sheep during gestation it has been possible to measure foetal arterial oxygen partial pressure (PaO_2) and arterial carbon dioxide partial pressure ($PaCO_2$). Foetal PaO_2 and $PaCO_2$ are usually 25% and 135%, respectively of the values in the conscious ewe [41;42]. Low PaO_2 and high $PaCO_2$ in the foetus exist because concentration gradients are required for these gases to diffuse across the placenta [43]. In mature mammalian animals and neonates, including humans, severe hypoxemia (PaO_2 below about 28 mm Hg) causes unconsciousness [10;44]. As PaO_2 is usually 20-27 mm Hg in foetal

Hg in foetal lambs, the foetal brain is exposed to an O_2 tension that would cause unconsciousness during postnatal life. This suggests that foetal consciousness or awareness may be suppressed by low O_2 status [10]. This proposition is supported by the finding that higher than normal foetal PaO_2 stimulated continuous breathing and behaviour arousal in foetal lambs [45;46].

Foetal catheterising has also made it possible to assess foetal concentrations of hormones. Progesterone, its metabolites and synthetic analogous have potent sedative and anaesthetic properties in adult animals including humans [47]. In the pregnant ewe the placenta produces large amounts of progesterone throughout the last half of the pregnancy, and especially during the last 20-30 days. Experimental injections of progesterone into the foetal circulation during the last 30 days of pregnancy reduce foetal EEG, breathing movements and behaviour arousal. In contrast, inhibition of placental progesterone production enhances the same parameters [48]. This suggests progesterone as a suppressor of fetal arousal.

There is also evidence that heat may suppress behaviour arousal. The foetus is slightly hyperthermic in relation to the mother because the heat production can only be dissipated down a thermal gradient across the placenta [49]. Foetal lambs cooled in utero exhibited arousal, shivering and increased respiratory activity whereas re-warming of those foetuses reversed the effects [49;50].

Experimental occlusion of the umbilical cord supplemented with adequate oxygenation, induced behaviour arousal and continuous breathing in foetal lambs. Infusion of a placental extract, but not vehicle, abolished this arousal and respiration within 2 minutes. This suggests that a placental factor, probably a protein, inhibits arousal during foetal life [51].

Based on evidence obtained from research on foetal sheep it appears that the lamb is not conscious or aware during gestation. This means that perceptual awareness may only be possible after the lamb has begun to breathe effectively and its arterial PaO_2 has risen significantly above foetal levels. If the foetal lamb is unconscious or unaware until after birth due to those parameters discussed above, it would indicate that the foetus exposed to painful stimuli during pregnancy or during birth is not able to feel pain [10]. At present, there are no comprehensive methods available that clearly prove that foetuses remain unaware and unable to experience pain throughout gestation. Nevertheless, it would be helpful in the future to test

for responsiveness in cortical regions by using neural imaging techniques. This could increase our understanding whether the foetus is conscious or not.

Conclusion

The current data on the development of the somatosensory pain system during foetal life indicate that pain pathways and cortical and sub cortical centres involved in pain perception are well developed late in gestation. Furthermore, the neurochemical systems associated with pain transmission are intact and functional. Theoretically, in humans, pain perception becomes possible following the establishment of the thalamo-cortical connection at about the beginning of the last trimester (gestational weeks 26-30). By careful extrapolation, this may also be valid for other mammalian species, such as some domesticated farm and laboratory animals. However, due to differences in foetal maturity between many of these animals and between some animals and humans, more research is needed to accurately evaluate the consequence of foetal maturity on pain perception differences in animal foetuses. Even though the systems for pain sensation and perception are in place, the foetus may not be able to feel pain because it appears to be unaware until after birth. A strong evidence for this hypothesis is that normal levels of O_2 in the circulation of the foetal lamb are below the concentrations required to support awareness in neonates and adults.

The other important issue related to prenatal stress is the increasing evidence for permanent developmental changes in response to noxious stimuli, and such stimuli may not need to penetrate consciousness in order to irreversibly alter central nervous development. Animal studies have demonstrated that painful stimulation is able to activate sub cortical mechanisms and mount different stress responses regardless of the cortex. This means that after establishment of the connection between the dermal nociceptors and the spinal cord at about week 10 in human foetuses, there are no safe periods during the gestation in which painful stimuli may be completely harmless. Knowledge on the association between foetal stress and developmental effects are still poor. However, studies have demonstrated that permanent changes due to painful stimulation may be prevented by adequate pain treatment. The current data indicate that treatment should be applied regardless of foetal age in mammalian species.

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Effects of prenatal stress on behaviour, physiology and morphology of offspring from mammals, birds and fish

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Introduction

The present report is a review of research on the effects of stress experienced by pregnant or mature females on various characteristics of their subsequent offspring. Farm animals may potentially experience several types of premating and prenatal stressors. Animals may be exposed to handling by humans in connection with mating or insemination and during gestation, inadequate and frustrative housing conditions, social stress related to dominant neighbours, crowding, or transport or being moved to another pen or cubicle with disruption of social contact and exposure to novel stimuli. These situations may not always cause stress, but may do so in some individuals. Stress during pregnancy has for a long time been known to affect embryonic survival and mortality, and is therefore important for the reproduction of farm animals. In this report focus will be on effects measured postnatally. There are some indications for long-term effects of prenatal stress on the survival, behaviour and physiology of farm animal offspring. The evidence presented highlights prenatal stress as a likely source of behaviour problems and welfare problems in farm animals, zoo animals, laboratory animals, and pets.

In the present context the stress concept is used quite broadly, disregarding short-term arousal effects. Prenatal stress may be defined as *stress experienced by the pregnant mother that affects the development of the offspring*. It is important to note that cognitive and emotional aspects of the stressor operate directly on the mother, but only indirectly on the fetus to the extent that such aspects could be mediated from mother to fetus. The ultimate emotional effects of the prenatal stressor may therefore differ between the mother and the offspring.

Prenatal stressors

A number of different types of stressors are used in research on prenatal stress. Pregnant mothers may be subjected to crowded conditions (Dahlöf et al., 1977), unstable social conditions (Sachser and Kaiser, 1996), social isolation (Roussel et al., 2004), handling by humans (Ader and Conklin, 1963), avoidance tests (Masterpasqua et al., 1976), conditioned avoidance (Thompson, 1957), REM sleep deprivation (Velazquez-Moctezuma et al., 1993), uncontrollable and unpredictable electric shocks (Takahashi et al., 1992a), flood light (Ward and Weisz, 1980), flashing lights and ringing bells (Weinstock et al., 1992), restraint (Jeppesen and Heller, 1986; McCormick et al., 1995), heat and restraint (Politch and Herrenkohl, 1984a), saline injections (Grimm and Frieder, 1987), or food restrictions (Wright et al., 1988). Some of these stressors may be present in routine management of farm animals.

The nature of the stressor may affect the results. Velazquez-Moctezuma et al. (1993) found different effects of four prenatal stressors on the sexual behaviour of male offspring: immobilization and deprivation of REM sleep impaired masculine sexual behaviour while immersion in cold water in fact facilitated masculine behaviour, only immobilization facilitated feminine sexual behaviour, whereas unavoidable electric foot shocks had only minor effects. Vinogradova et al. (1996) found different effects on the rat offspring of pain experienced and pain observed in mates (regarded as a psychological stressor) by their pregnant mothers. While Takahashi et al. (1990) recorded a reduced frequency of ultrasonic vocalizations during isolation in young rats (14 days old) exposed prenatally to uncontrollable electric shocks, Williams et al. (1998) reported increased number of such vocalizations at the same age after exposure to prenatal heat-light-restraint stress.

Gestational periods

In most of the studies on prenatal stress the stressor is given during the last third of the pregnancy (third trimester), usually as daily treatments. There is however a stress-sensitive period during the very first days of pregnancy, especially until implantation of the fetus (Von Borell, 1995). In a rat study comparing the effects of REM sleep

deprivation during either the first, second, or third trimester, different effects appeared in the different trimesters (Suchecki and Neto, 1991). The results suggested that open-field ambulation of adult male offspring was most affected by prenatal stress in the first trimester, whereas an anxiogenic drug affected adrenal weight of offspring mainly when given during the second trimester. In a study on squirrel monkeys, the neuromotor development in offspring was retarded if the mother experienced repeated disruptions of her social relationships throughout gestation, but not if she experienced this only once during mid gestation (Schneider and Coe, 1993). To summarize so far, both the type of prenatal stressor and the period it is administered may influence the effects on the offspring. The mechanisms responsible for these differences are unknown.

Effects on behaviour of offspring

Locomotion, exploratory behaviour, play, and fear of novelty

Prenatally stressed monkeys may show retarded motor development. Infant squirrel monkeys, whose mothers had experienced repeated disruption of social relationships throughout pregnancy, had poorer motor abilities, impaired balance reactions, and shorter attention and looking episodes when given orientation items (Schneider and Coe, 1993). Rhesus monkeys prenatally stressed with daily unpredictable noise stimuli and tested at one month of age showed lower muscle tones, poorer coordination, slower response speeds, delayed self-feeding, and were more distractible than controls (Schneider, 1992a). At six months these monkeys showed lower levels of gross motor behaviour and exploration in a novel environment. Especially the males showed more clinging to surrogates instead (Schneider, 1992b).

Prenatally stressed rats have several times been reported to show suppressed exploratory behaviour in a novel environment, which may be indicative of increased fearfulness. In his seminal work forty years ago Thompson (1957) stressed pregnant hooded rats by thwarting them from an escape opportunity which was previously learnt by conditioned avoidance. Their adult offspring showed a tripled latency to move and a pronounced reduction in distance run in an open field. This was stable when retested 100 days later. In another test after food deprivation, the prenatally stressed rats showed

stressed rats showed a longer latency to leave their cages and reach food at the end of an alley. In a study adopting the same methods, it was found that prenatally stressed Sprague-Dawley rats in contrast showed increased exploration in an open-field and spent a greater amount of time outside their home cage when allowed (Masterpasqua et al., 1976). This might suggest differences in behavioural strategy between selection lines of rats. It also reminds us that various motivational systems may underlie activity in an open-field test (Denenberg, 1969): general exploratory behaviour, specific appetitive behaviour, and fear-induced flight or escape. By use of a principal-component analysis on three different tests (Y-maze, open-field, and elevated plus-maze), Vallée et al. (1997) found low exploration, but more pronounced escape behaviour in Sprague-Dawley rats prenatally stressed with restraint-and-light compared to controls. In another study, adult rats prenatally stressed unpredictably with noise and light three times weekly showed a reduced number of arm entries in a plus-maze (with two walled arms and two open arms), reduced time spent in the open arms, and reduced locomotion and rearing in a well-lit open field (Poltyrev et al., 1996). Rats prenatally stressed with noise and light made fewer centre entries in an open field and deposited more fecal pellets (Weinstock et al., 1992).

At 35 days of age, prenatally stressed blue-fox cubs showed increased reactivity in three tests for response to novelty compared to control cubs; increased activity in an open-field test, more frequent re-entry into the open-field from a dark box, and more persistant activity when being held by a human (Braastad et al., 1998). Prenatally stressed lambs showed increased basal cortisol concentrations, and more exploration and locomotion in behavioural tests at 8 months of age but not at 25 days of age (Roussel et al., 2004). At four years of age, prenatally stressed rhesus monkeys showed more locomotion and disturbance behaviour when separated from cagemates and grouped with unfamiliar animals (Clarke et al., 1996). They played only one sixth of the time control animals played. In a special playroom the prenatally stressed monkeys showed more inactivity and less exploratory behaviour, and increased distress vocalizations, compared to controls.

An increased frequency of defensive freezing induced by electric shocks is reported in young and adult rats exposed to prenatal stress (Takahashi et al., 1992b).

Effects of prenatal stress on emotionality are sometimes lower in adult or old animals than in younger ones. Compared to control animals, Takahashi et al. (1990) recorded a reduced frequency of ultrasonic vocalizations and lower increase in tail-flick latencies during isolation in young rats (14 days old) exposed prenatally to uncontrollable electric shocks, but no difference was found in juvenile rats (21 days). Batuev et al. (1996) reported lower locomotion and higher anxiety in prenatally stressed rats at 1 month, but not at 4 months.

Also in humans effects of prenatal psychological stress (e.g. unpredictable aircraft noise, threat of impending war, or marital problems) are reported in children, who showed delays in early motor development and increased frequencies of behavioural abnormalities like excessive clinging, crying, hyperactivity, and low frustration threshold, as reviewed by Weinstock (1997).

Learning ability

Prenatally stressed animals have been subjected to several learning tests. When tested in a water maze twice a day (Thompson et al., 1962), it is generally found that prenatally stressed rats need increased time and have higher error scores before they learn the maze (Archer and Blackman, 1971). The opposite has also been found, in animals tested five times during one day (Morra, 1965). In conditioned avoidance tests prenatally stressed rats showed shorter latencies and more avoidance responses than controls (Joffe, 1965a,b). This might reflect greater fear or anxiety, which facilitates the learning of avoidance responses (Archer and Blackman, 1971).

Smith et al. (1981) conducted several learning tests (discrimination learning, T maze, and runway) on the offspring of rat mothers stressed by either conditioned shock avoidance or handling only. The handled and stressed groups were inferior to an unstressed control group on four of the six learning measures. When tested for complex discrimination learning of brightness in a maze, prenatally stressed rats learnt more slowly than controls (Grimm and Frieder, 1987). In a delayed-response test, a kind of Piagetian object permanence task (Goldman-Rakic, 1992), prenatally stressed rhesus monkeys took longer than controls to locate an object that was partially obstructed or observed to vanish from view (Schneider, 1992c). The author concluded that the

that the prenatally stressed monkeys were cognitively impaired. Performance of the object permanence function is associated with maturation of working memory in the brain's prefrontal cortex. In general, it seems that prenatal stress may impair learning ability, but may facilitate learning in fear situations.

Social behaviour and aggression

The latency to show social play was higher in prenatally stressed sibling pairs of 4-week-old rats than in controls, but after initiation no difference in frequency of the play was found (Takahashi et al., 1992a). When electric shock was given on one test day, defensive freezing was more frequent among the prenatally stressed rats, although both groups showed the same decline in social play. Aggressive behaviour is reported to be more expressed in prenatally stressed rats than controls, regardless of sex (Batuev et al., 1996). In another study, prenatal stress (bright light and heat) tended to reduce the propensity of female rats to exhibit aggression towards an intruding female (Vom Saal et al., 1991).

Prenatally stressed rhesus monkeys (18 months of age) showed more mutual clinging and less normal proximity and social contact during stressful conditions than controls (Clarke and Schneider, 1993). Similar results were found at four years of age (Clarke et al., 1996).

These results may indicate that prenatal stress impairs social behaviour mainly through increased fearfulness and a more defensive behaviour. In a study on pigs, de Jonge et al. (1996) observed increased susceptibility to social stress among animals kept in a standard poor environment compared to controls kept with access to outdoor pasture. As the environmental conditions were constant during gestation and lactation, the authors regarded prenatal tethering stress as one possible mechanism behind the results, although postnatal effects could not be excluded.

Sexual behaviour, reproductive success, and maternal behaviour

Male offspring

Prenatal stress is shown to have pronounced effects on sexual behaviour in male and female rodents, although the effects may sometimes vary with the type of stressor

(Velazquez-Moctezuma et al., 1993; see section 1.2). Ward (1972) reported prenatally stressed male rats to have low levels of male copulatory behaviour and high rates of the female lordosis response, indicating both a demasculinization and a feminization of these males. These effects were shown after either nutritional stress, complex environmental stress, or ACTH injections during pregnancy (Rhees and Fleming, 1981). Reduced male copulatory behaviour, but no effect on lordosis, was found after prenatal ACTH injections in mice (Politch and Herrenkohl, 1984b). Low levels of male copulatory behaviour in short-term tests may develop into successful impregnation of females during long-term tests (Masterpasqua et al., 1976). Other studies found increased readiness for lordosis but no reduction in masculine sexual behaviour after prenatal stress (Dahlöf et al., 1977; Politch and Herrenkohl, 1984a).

The demasculinization and feminization of male rats after prenatal stress has been suggested to be related to an absence of the increased testosterone production which normally occurs in the fetus during the days before birth (Ward and Weisz, 1980). These effects have been shown to be prevented by perinatal androgen treatment of the offspring (Dörner et al., 1983).

Female offspring

Also in female offspring pronounced effects of prenatal stress on sexual behaviour and reproduction are reported. Herrenkohl (1979) observed higher rates of failure to become pregnant, more spontaneous abortions, longer pregnancies, and offspring lighter in weight and less likely to survive the neonatal period in prenatally stressed rats. Delayed sexual maturation, but longer estrus cycles and a higher degree of receptivity are reported in prenatally stressed mice (Politch and Herrenkohl, 1984a). The inhibitory effect on length of the first estrus cycle of group-housed female mice which developed between two female fetuses decreased if they were subjected to prenatal stress (Vom Saal et al., 1991). Female guinea pigs whose mothers experienced an unstable social environment during pregnancy, showed more male-typical courtship behaviour, play, and social orientation than controls (Sachser and Kaiser, 1996). Prenatally stressed rats showed normal maternal behaviour under normal conditions but a marked reduction in pup retrieval compared to controls in a conflict situation in

conflict situation in which they had to pass through an airstream (Fride et al., 1985). This suggests a lower motivation to retrieve. In another study prenatally stressed adult male and female rats were exposed to young rat pups (Kinsley and Bridges, 1988). The females exhibited a longer latency than controls to show full maternal behaviour including pup retrieval, crouching over pups, and nest building. In males, however, the latency to show full maternal behaviour was shorter than in control males. The authors concluded that prenatal stress made the females more male-like and the males more female-like.

Positive effects of mild postnatal glucocorticoid exposure

Generally, studies show that prenatal stress might cause permanent detrimental effects on physiology and behaviour in offspring. Interestingly, research provides evidence that mild postnatal stressors might instigate quite opposite, beneficial consequences relating to stress coping, fearfulness and learning ability. An experiment by Catalani et al. (2000) demonstrated that when the increment of glucocorticoids during infancy is moderate, rat offspring had increased number of glucocorticoid receptors at 30 days of life as well as lower hormonal responses to stress and better performance in a spatial memory test at three months of age. Moreover, adult rats exposed to mild prenatal stress show reduced anxiety, improved learning and a better coping strategy in stressful environments (Casolini et al., 1997; Catalani et al., 1993). Hence, glucocorticoid-induced early events have impacts on physiology and behaviour in adulthood, and these consequences may be beneficial or detrimental depending on plasma levels of glucocorticoids, as well as on the nature of the stimulus and the developmental stage at which the experiences the event.

Neuroendocrine mechanisms of prenatal stress

Neuroendocrine effects on offspring

The best documentation of prenatal stress effects on the physiology of the offspring concerns the development of responsiveness to stress and novel stimuli being related to developmental effects on the hypothalamic-pituitary-adrenocortical (HPA) axis. In this

this axis corticotrophin-releasing hormone (CRH) and vasopressin (AVP) are secreted by the hypothalamus and stimulate the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH stimulates the synthesis and release of glucocorticoids, including cortisol and corticosterone (rodents), by the adrenal cortex. Altered responsiveness to stress in the HPA axis after prenatal stress is frequently reported in adult offspring of rats, the direction of the effect is not clear however. Some report increased responsiveness (e.g. Takahashi and Kalin, 1991; Henry et al., 1994) while others report decreased responsiveness (e.g. Naumenko and Maslova, 1985; Fameli et al., 1994). It is concluded that prenatally stressed animals show impaired coping in stressful situations, and that this is caused, at least partly, by a dysregulation of the HPA axis characterized by decreased feedback inhibition of CRH and prolonged elevation of plasma corticosteroids (Weinstock, 1997). This reduced negative feedback is shown to be due to downregulation of glucocorticoid receptor gene expression in hippocampus and frontal cortex (Meaney et al., 1996) leading to a decrease in hippocampal corticosteroid receptors (Barbazanges et al., 1996). The elevated circulating corticosteroids increase CRH in amygdala, at a site which is implicated in the production of fear and anxiety (Weinstock, 1997). Interestingly, these effects on the HPA function are quite opposite to those found in animals which are exposed to postnatal human handling with maternal deprivation (e.g. Ogawa et al., 1994) or increased maternal care (Liu et al., 1997). The HPA effects of prenatal stress in rats somewhat parallel the HPA function observed in depressed humans (Checkley, 1996; Weinstock, 1997). If this reflects general effects across species, it might be interesting to study the relation between prenatal stress and behavioural depression in farm animals.

Mediation of prenatal stress from mother to fetus

One mediating route of the prenatal stress between mother and fetus is well-documented and involves maternal glucocorticoids (Rhees and Fleming, 1981; Politch and Herrenkohl, 1984b; Götz et al., 1986; Williams et al., 1995). Prenatal stress is mediated through the transplacental crossing of glucocorticoids from the mother to the fetuses (Zarrow et al., 1970; Barbazanges et al., 1996), at least in the last third of the

gestation (ACTH, a large peptide molecule, does not cross the placenta). In late gestation of rats the fetal HPA axis and the negative feedback mechanism of glucocorticoids in the fetal brain have been shown to be functioning (Dupouy and Chatelain, 1984). In silver fox embryos ACTH-stimulated cortisol production is recorded already 15 days prior to birth (Osadchuk, 1997). Hyperproduction of glucocorticoids in stressed females may therefore affect the development of embryonic adrenal function in their offspring. If the fetus receives high levels of glucocorticoids during late gestation, it may be irrelevant whether the environmental effects on the mother satisfy rigid definitions of stress (e.g. Broom and Johnson, 1993).

It is unknown to what extent group variations recorded in prenatal stress research could be explained solely by variation in glucocorticoid secretion in the pregnant mother or some other hormones related to the mother's emotional experience. In a rat study with prenatal exposure to either ACTH, corticosterone, dexamethasone (which blocks the release of ACTH and endorphins from the pituitary), or restraint-and-light stress, the traditional effects on male sexual behaviour was found in the dexamethasone and stress groups, but not in the ACTH or corticosterone groups even when their mothers showed a resulting higher plasma corticosterone level than the stressed mothers (Holson et al., 1995). This suggests that the HPA axis may not be the only neuroendocrine system mediating effects of prenatal stress. It is quite possible that different prenatal stressors affect different neuroendocrine pathways. Velazquez-Moctezuma et al. (1993) speculated whether the differential effects of several types of prenatal stressors on male sexual behaviour (see section 1.2), in addition to corticosterone, could be related to the effects of opioids, noradrenalin and serotonin. These hormones might mediate emotional aspects of the stressor. The results of several studies suggest that excessive β-endorphin in the stressed mother may cross placenta and mediate feminization of male sexual behaviour (Weinstock, 1997). It is also possible that some of the stressor effects are related to a weight loss sometimes seen in the mothers (Ward and Wainwright, 1988).

Prenatal stress in fish

Farmed fish are exposed to a wide range of temporary and chronic challenges during various phases in their life cycle, and the conditions to which fish farm broodstock are subjected during sexual maturation might be a significant factor in determining the nature of gametes produced and the succeeding offspring characteristics. Common procedures in hatcheries such as transportation, handling, cleaning and crowding, as well as problems with water quality are stressors that may negatively influence reproductive mechanisms. In teleost fish the maternal endocrine system and that of the progeny is closely associated, and current evidence suggests that diverse developmental, reproductive and metabolic hormones are released into the nutritive yolk sac during oogenesis in quantities that reflect female plasma levels (Schreck et al., 1991). The physiological state of the mother during gametogenesis thus molds the provisioning of her eggs, and events that affect the hormonal regime of the mother may have downstream effects on subsequent brood.

A few experiments have illustrated detrimental consequences of parental stress on offspring characteristics in fish. Deleterious effects of maternal stress on progeny survival are reported (Campbell et al., 1992, 1994). In addition, McCormick (1998) found that mothers with increased plasma cortisol levels produced alevins that were smaller and had reduced yolk sac size. Campbell et al. (1992, 1994) and Contreras-Sanchez et al. (1998) showed that exposure of cultured rainbow trout to acute and chronic stressors before spawning resulted in smaller swim-up fry compared with controls. Ostrand et al. (2004) reported that age-0 largemouth bass produced from parents exposed to stress were smaller and weighed less, and had later swim-up dates than did controls. Until now, little have been known regarding how prenatal stress might affect the ability of teleost offspring to cope with a stressor later in life. However, recent research by Eriksen et al. (2005) demonstrated that maternal cortisol challenge late in oogenesis have the potential to impair the ability of progeny to cope with an environmental challenge, exemplified by an episode of hyperthermia. This was reflected by increased offspring mortality, reduced alevin length and body weight, diminished yolk sac volume and decelerated yolk sac utilization.

Within commercial aquaculture, specimens inflicted with various morphological anomalies are a recurring problem. A great number of farmed fish show morphological deformities, involving skeletal malformations, organ anomalies, scale disorientation and bodyline irregularities. The aetiology of these syndromes is not fully elucidated, however, a wide range of biotic and abiotic factors such as thermal or mechanical shocks, pollutants, high density, nutritional deficiencies, parasites, radiation and

radiation and oxygen depletion are cited to cause aberrations in development (Divanach et al., 1996). It is also shown that increased levels of maternal cortisol may enhance the frequency of deformed offspring (Eriksen et al., 2005). Hence, the high occurrence of malformations in aquaculture might thus also partially reflect suboptimal parental husbandry conditions.

In conclusion, studies into prenatal stress in teleosts accentuates the importance of increased knowledge on how the maternal endocrinological state during gametogenesis may impinge on offspring characters in farmed fish, and further substantiates the necessity of incorporating maternal effects when evaluating rearing conditions and animal welfare within commercial aquaculture.

Prenatal stress in domestic fowl

Stress in hens may affect the hormone content of eggs and lead to prenatal stress also in birds. The effects of prenatal stress in hens can be studied by comparing birds produced from the eggs of stressed and unstressed hens (Janczak et al., 2005a). It can also be studied by comparing birds from eggs with artificially elevated levels of corticosterone with birds from normal eggs (Janczak et al., 2005a). A few recent studies have been based on the comparison of normal chicks (control) with chicks that received corticosterone injections in the egg prior to incubation (treatment). The concentration of corticosterone injected into treated eggs was similar to the mean concentration in the blood of stressed hens. Recordings included effects on behaviour and growth (Janczak et al., 2005b; Nordgreen et al., 2005). The ability to compete for resources is important for loose-housed hens. Birds with a reduced ability to compete may have reduced access to resources such as feed and perches. The relative ability of treated and control chicks to monopolize a model worm that looked like an attractive edible object was therefore recorded to test for effects of the treatment on competitive ability. Chicks from eggs with no added corticosterone won significantly more in this competitive situation (Janczak et al., 2005b). A similar measurement in mature laying hens competing for feed indicated that this effect of corticosterone was stable (personal communication). Cognitive ability is important in alternative production systems. Birds that have difficulty in learning about and remembering how to find feed, water,

feed, water, perches and nest boxes will not function well. Learning and memory was tested in chicks from the control and treatment groups by exposing chicks to artificially coloured flock members (Nordgreen et al., 2005). Their preference for a chick of a familiar or unfamiliar colour was then recorded in a preference test that measured their ability to discriminate between the two. Control chicks were significantly better than treated chicks at discriminating between familiar and unfamiliar stimulus chicks (Nordgreen et al., 2005). This finding was later confirmed in another experiment using coloured objects instead of coloured chicks as stimulus objects (Nordgreen et al., 2005). In addition to learning and remembering the characteristics of the home environment, the ability to cross barriers to access resources is also important for welfare and productivity. This is especially true in systems such as aviaries where resources are distributed over several levels. The chick's ability to cross a barrier in order to access feed on the other side was therefore also tested. Chicks were placed on one side of a wire partition and their feed was placed on the other side. The number of chicks having crossed the wall to the feed at successive 15-minute intervals was then recorded. Control chicks were significantly better at solving this problem (Janczak et al., 2005b). Fear of humans is important because fearful birds are more predisposed to stress and hysteria, both of which are associated with reduced welfare and productivity. The last behavioural registration therefore involved the observation and comparison of fear in treated and control chicks. Fearful birds avoid humans whereas less fearful birds may to a higher degree approach humans. To register fearfulness an observer sat in the chick's home cage and recorded the number of birds within 50 cm. In this situation there was also a significant difference between the treatments, corticosterone birds showing higher fear of the human in their home cage (Janczak et al., 2005b). This finding was later repeated in another independent experiment (Janczak et al., personal communication). Recordings of growth indicated a negative effect of corticosterone treatment at 1 and 4 weeks of age (Janczak et al., 2005b), but not in mature laying hens (personal communicaton). There was no effect of the treatment on damage caused by pecking in chicks or adult laying hens (*ibid*).

Relatively small changes in the production environment or daily routines can cause stress for laying hens living in an otherwise stable production environment. Examples are changes in feed quality or access to feed, and lighting. Hormone samples from hens exposed to an unpredictable feeding regime (stressed) and control hens were compared to test for effects on corticosterone concentration in incubating eggs and corticosterone metabolites in faeces. Preliminary results from these experiments indicate that chronic stress does indeed lead to a significant increase in the concentration of corticosterone metabolites in hen faeces but did not influence the corticosterone content of eggs in this experiment (Janczak et al., 2005c). However, despite the lack of effects on egg hormone content the progeny of hens subjected to unpredictable feeding were found to have a reduced ability to compete and a higher level of fear in response to handling by humans (Janczak et al., 2005c). This indicates that other hormones such as testosterone, estradiol and androstenedione may also mediate effects of prenatal stress in hens and will be studied in further experiments (Janczak et al., 2005c). Although no effect of the feeding treatment was found, hens subjected to injections of corticosterone while in the egg had the higher corticosterone content in their own eggs when subjected to the unpredictable feeding treatment (personal communication).

To summarize, experiments on prenatal stress in hens indicate that chicks hatching from eggs with elevated levels of corticosterone have a reduced ability to learn or remember other individuals and objects, a reduced ability to cross barriers to access resources such as feed, a reduced ability to compete, an elevated fear of humans, an elevated stress sensitivity and a reduced growth rate. It is also shown that prenatal environmental stress has effects on competitive ability and fear in hens. The results imply that stress in laying hens and elevated levels of stress hormones in incubating eggs may have a number of negative effects on the welfare and productivity of chickens living in alternative production systems. The main benefit of these findings for the industry is that a larger amount of the observed variation in behavioural problems in laying hens can now be explained by stress in hens producing incubating eggs. This should result in an increased motivation to reduce stress in layers that produce eggs for hatching purposes and to improve the early environment for chicks.

Prenatal stress and animal welfare

Some of the observed relations between prenatal stress, neuroendocrine development, and adult behaviour may have implications for animal welfare. Prolonged or increased HPA activity in novel or stressful situations, with accompanying increased fearfulness - whether this suppresses or activates behaviour - indicates reduced welfare. Such effects could arise from a number of treatments normally experienced by farm animals including fish. Unpredictable and uncontrollable handling prior to or during gestation, and being moved to another pen or crate with disruption of social relations, might be postulated to induce the most marked effects, since habituation to the treatments would be unlikely.

It is also possible that moderate prenatal stress may have positive and adaptive effects on the development of the HPA axis and its related brain mechanisms, by allowing these systems to be tested and calibrated to the existing environmental conditions. Individual variation might be expected as to how much endocrine stimuli could be processed in the fetus before negative effects develop.

In rhesus monkey infants, prenatal stress is reported to induce more stereotypic behaviour in a stressful environment (Schneider, 1992b). Humans that have experienced prenatal stress may have a lower threshold for becoming frustrated, and humans with endogenous depressions show a similar functioning of the hippocampal-HPA axis as in prenatally stressed animals (Checkley, 1996; Weinstock, 1997). These observations may be interesting with regard to frustration-related behavioural disturbances in confined animals, like e.g. stereotypies, restlessness, redirected behaviours, overflow activities, and vacuum activities. It might also be speculated whether learned helplessness is more likely to develop in prenatally stressed animals. We suspect that the development of behavioural disturbances in farm animals on land and in water (or lab, zoo, and pet animals) will be better understood if prenatal, or even premating, stress is considered.

Conclusions

Evidence mainly from studies of rodents and primates strongly indicate that prenatal stress can impair the stress-coping ability of juvenile and adult offspring and disrupt their behaviour in aversive or conflict-inducing situations (Barbazanges et al., 1996;

Weinstock, 1997). Effects may be found on their sex-ratio at birth, on locomotion, play, exploratory behaviour, fearfulness, learning ability, social behaviour, aggression, sexual behaviour, and maternal behaviour, and on their reproductive success in the first, and sometimes also in the second, generation. In normal situations without challenge, behavioural effects of prenatal stress are frequently not seen. Individual variation in the susceptibility to prenatal stress may exist. Behavioural inhibition and anxiety when exposed to novelty are typical results which may underlie the effects of prenatal stress on learning and various behavioural responses. This seems to be related to increased or prolonged activity in the HPA axis produced by impaired negative feedback of glucocorticoids in the hippocampus, although several other neuroendocrine pathways may be involved.

Since behavioural and neuroendocrine effects of prenatal stress in rodents are quite similar to those found in depressed humans, and since increased fearfulness and frustration is implicated, it may be predicted that farm animals subjected to prenatal stress will show a reduced ability to cope with a difficult environment and have an increased propensity for developing behavioural disturbances and reduced welfare. Recent results on farmed foxes, and indications in other farm species, show that prenatal stress may affect the behavioural development of farm animals.

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