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BEHAVIOURAL STATE CYCLING AND RELATED CHANGES IN THE CEREBRAL BLOOD FLOW IN THE OVINE FETUS NEAR TERM

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ABSTRACT

The low voltage (LV)/rapid eye movement (REM) behavioural state the high voltage(HV)/non-rapid eye movement (NREM) behavioural state are each suggested to have a unique functional role in fetal neurodevelopment, therefore requiring the existence of both behavioural states in appropriate proportions for optimal maturation. The present study examined the behavioural state cycling pattern in the ovine fetus near term and characterized the related changes in cerebral blood flow velocity (CBF_v), utilizing a 20-MHz piezoelectric Doppler crystal transducer. Our results demonstrated the HV/NREM epoch duration to be positively correlated with that of the prior LV/REM epoch, as well as with that of the subsequent LV/REM epoch, suggesting a possible homeostatic behavioural state control mechanism in the fetus. Changes in CBF_v were consistent with those previously demonstrated, suggesting the piezoelectric Doppler crystal transducer may be used to continuously measure CBF_v under resting conditions and provide a relative measure of cerebral blood flow changes.

Key Words: fetal behavioural state, sleep state cycling, behavioural state linkage, behavioural state epoch duration, cerebral blood flow, piezoelectric crystal transducer

CO-AUTHORSHIP

The following people contributed to the manuscripts contained within this thesis in the following ways:

Dr. B Richardson: Supervisor throughout all projects, provided grant funding to complete experiments, edited manuscripts

Dr. M Czikk: Performed sheep surgeries and experiments, provided input into data analysis interpretation

Ms. A Keen: Provided input into data analysis interpretation, edited manuscripts

Dr. M Frasch: Provided input into data analysis interpretation, edited manuscripts

Ms. S Hemstreet: Provided technical assistance during all surgeries and experiments

Mr. B Matushewski: Provided technical assistance during all surgeries, input into data analysis, edited manuscripts

To my parents

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LIST OF ABBREVIATIONS AND SYMBOLS

~ = approximately

A_{2A} = A_{2A} adenosine receptor

ATP = adenosine triphosphate

CBF = cerebral blood flow

CBF_v = cerebral blood flow velocity

CMR = cerebral metabolic rate

CNS = central nervous system

CPA = cyclopentyladenosine

D_{ss} = diameter estimate of sagittal sinus vessel

DNA = deoxyribonucleic acid

ECOG = electrocorticogram

EEG = electroencephalography

EMG = electromyography

EOG = electro-oculography

F = fetal

FBM = fetal breathing movement

FV = flow velocity

HV = high-voltage

Hz = hertz

I_{min} = minimal insonation depth

I_{max} = maximal insonation depth

I_p = peak insonation depth

ID = indeterminate state activity

IV = intermediate voltage

LV = low voltage

mm/sec = millimeters per second

NREM = non-rapid eye movement

pCO_2 = partial pressure of carbon dioxide

PaO_2 = partial pressure of oxygen

PET= positron emission tomography

REM = rapid eye movement

SCN = suprachiasmatic nucleus

SEM = standard error of the mean

μV = micro-volts

Chapter 1

LITERATURE REVIEW

1.1 BEHAVIOURAL/SLEEP STATE ACTIVITY

1.1.1 Adult sleep states

At one time thought to be a passive and homogenous state of inactivity, physiologists now view sleep as an extremely advanced and synchronized behavioural state in the mammalian species (1). Studies in the human adult have demonstrated that sleep in occurs in two distinct phases. Termed "sleep states," these phases display temporal patterns in a range of electrophysiological and behavioural parameters that repeat themselves over time and are relatively stable (2). Using measures in electroencephalography (EEG), electrooculography (EOG) and electromyography (EMG), criteria were designed such that these two alternating behavioural phases of adult sleep were classified as non-rapid eye movement (NREM) sleep state and rapid eye movement (REM) sleep state (3). The presence of these two sleep states appears to be species specific. For example, while most mammals appear to demonstrate both sleep states, the adult bird only displays a rudimentary form of the REM state and reptiles demonstrate almost exclusively NREM sleep (*as reviewed in* 4).

In the adult human and primates, NREM sleep, which is also termed slow-wave sleep, can be further classified into a series of stages ranging from NREM sleep stage I to NREM sleep stage IV, where the sequential succession from stage I to IV corresponds to a progressive decrease in consciousness and increase in EEG synchronization (3).

Specifically, there is a progressive decrease in EEG frequency and corresponding increase in EEG amplitude with each transition between stages. Stages III and IV are termed deep slow wave sleep and are identified by the total absence of eye movements.

REM sleep, which is also termed paradoxical sleep or active sleep, is identified by EEG desynchronization in which there is an increase in EEG frequency, a decrease in EEG amplitude, the presence of ocular saccades (i.e. rapid eye movements), and the absence of postural muscle movement. REM sleep may also be subdivided into phasic and tonic REM sleep, whereby phasic REM distinguished by a relatively high amount of eye movements and gross body muscle twitches as well as phasic central nervous system (CNS) electrical events (5). The observation of postural muscle twitches accompanying EEG and EOG patterns similar to those seen during REM sleep indicate that organism is experiencing wakefulness. Therefore, the adult organism will alternate between periods of wakefulness and sleep, and within sleep, cycling between NREM sleep episodes and REM sleep episodes.

The cyclic organization of sleep varies within and between species. In the adult human, there is an alternation between the two sleep states that allows for approximately four to six sleep cycles in one night (1). The mature sleep/wake patterns in the human, which are displayed by 6 years of age (6), are suspected to originate from developmental precursors known as behavioural states exhibited by the fetus and neonate.

1.1.2 Neonatal sleep states

In the human neonate, sleep states can often be used to assess neurological maturation of the infant (4). While there are a number of neonatal sleep state classification methodologies that have been used in previous studies, the most widely accepted classification system is that proposed by Prectl, which rates parameters that are both easily observable and continuously evident (7). Neonatal characteristics identifying State 1 include closed eyes, the absence of gross body movement and normal breathing. This state is considered to be neonatal quiet sleep and resembles the NREM sleep state in the adult human. Neonatal sleep state 2 is characterized by closed eyes, irregular breathing, and infrequent gross body movements. This state is often referred to as active sleep and is similar to the REM state observed in the adult human. Prectl's classification of neonatal sleep states includes three additional states (States 3-5) in which the eyes are now open and the neonate is in wakefulness. While still absent during State 3, the presence of gross body movements become evident in State 4 and increase in abundance in State 5, with State 5 also characterized by crying (7). Prectl's identification of neonatal sleep states is determined, in large part, by observable changes in behavioural activity and therefore these definitions of neonatal sleep states allowed for the emergence of the concept of fetal behavioural states.

1.1.3 Fetal behavioural states

The concept of behavioural states in the fetus are similar to those used to characterize sleep in the adult and neonate and are defined as time periods in which there is synchronizing of several distinct electrophysiological and biophysiological parameters, with these periods cycling over time (4). The development of fetal behavioural states coincides with the organism's period of rapid brain growth and maturation, and therefore emerges in a species-specific manner (8). Well-defined behavioural states are present before birth in those species classified as prenatal brain developers (i.e. the ovine fetus) and perinatal brain developers (i.e. the human fetus). In these types of brain developers, a significant amount of neurodevelopment, which may include increases in brain weight, DNA synthesis, cell size, and myelination, occur before birth. In contrast, in species classified as postnatal brain developers (i.e. the rat, cat, and rabbit), in which these events tend to occur after birth, coordinated sleep-wake patterns are not evident until the postnatal period (4).

The criteria defining human fetal behavioural states, developed by Nihjuis, resulted in States 1F to 4F (F designating fetus) that are identified using ultrasound and simultaneous fetal heart rate recordings (9). The human fetus displays distinct fetal behavioural states at ~36 weeks gestation (10). These criteria were developed from those used to classify human neonate behavioural states (11). State 1F is analogous to State 1

in the newborn and NREM sleep in the adult, in which there is the presence of a stable heart rate and the absence of eye movements. State 2F is analogous to neonatal State 2 and REM sleep in the adult in which rapid eyes movements, frequent gross body movements and heart rate accelerations are observable. States 3F and 4F both resemble periods of wakefulness, with the incidence of eye movements increased during State 3F and both eye and gross body movements increased during State 4F (9). There are additional behavioural parameters shown to be associated with specific fetal behavioural states, though not continuously present and therefore are not considered state-defining features. These parameters include fetal breathing activity, which increases in its presence during 2F compared with 1F and bladder emptying, which appears to increase during periods of rapid eye movements (i.e. 2F or 4F).

The current model used for the study of fetal behavioural states is the chronically catheterized ovine fetus, which displays well-coordinated behavioural states at ~120 days gestation (term =145 days). This model is utilized, primarily because it allows for the invasive monitoring of behavioural state parameters and its associated physiological activities as well as its allowance of manipulation of the fetal environment. Additionally, the ovine fetal model has been utilized in fetal behavioural state studies due to its similarity to the human fetus with respect to the development and emergence patterns of behavioural state activity (8). Behavioural state studies utilizing the ovine fetal model record the following state-

defining parameters: electrocortical (ECOG) activity from the parietal cortex, electroocular (EOG) activity recorded from the lateral canthus of the eye and electromyographic (EMG) activity of the nuchal neck muscle or chin using polygraph recordings (4). Measurements from these three parameters in the ovine fetus allow for the observance of three distinct states of behavioural state activities that are similar to the sleep states seen in adult.

The first is termed the LV/REM state, which is characterized by low voltage (LV) high frequency ECOG activity, the presence of rapid eye movements (REM) and the absence of nuchal muscle activity. The LV/REM state is similar to the REM state observed in the adult. The second behavioural state is termed the HV/NREM state is characterized by high voltage (HV) low frequency ECOG activity, the absence of rapid eye movements (NREM) and the presence of nuchal muscle movements. This state resembles the adult NREM state. Collectively, these two states are considered to represent *in utero* sleep state activity given their similarity to neonatal sleep states (4). The third behavioural state activity resembles postnatal wakefulness and is characterized by low voltage ECOG activity, the possible presence of eye movements and the presence of nuchal muscle tone.

Once the ovine fetus develops mature and well-coordinated behavioural states at ~120 days gestation, it will spend approximately 90% of its total time in either the LV/REM state or the HV/NREM state (12). The

remaining 10% of its total time is compromised of wakefulness as well as short periods of transition time in which the fetus alternates from one behavioural state to another behavioural state (i.e. often occurring for less than 2 minutes) (12).

As in the human fetus, additional physiological parameters in the ovine fetus exhibit distinct changes associated with the presence of a particular behavioural state, although they are not required for the definition of that state. As is the case with behavioural states themselves, the presence and characteristics of these associated parameters are specific to the species. For example, fetal breathing movements (FBM) are frequently associated with behavioural state activity. In the ovine fetus, FBM will coordinate with the cycling of the LV/REM and HV/NREM states upon the maturation of the temporal relationship between the defining behavioural state parameters, with FBM occurring approximately 60% of the time during LV/REM state with periods of apnea evident during the HV/NREM state by 125 days gestation (13). Since FBM occurs irregularly, with one-third of the LV/REM state occurring without their presence, they therefore cannot be used as a criterion for identifying the LV/REM state. The ovine fetus has also demonstrated an associated increase in heart rate during the HV/NREM state, possibly mediated by the increase in gross body movements observed during the HV/NREM state (14-16). In the ovine fetus, the activities of repeated swallowing and

bladder contractions also appear to be state dependent, with these activities occurring mainly during wakefulness (17, 18).

1.1.4 Ontogeny of behavioural/sleep state activity

Studies investigating the temporal relationship between the behavioural and physiological parameters have confirmed that not only are fetal behavioural states similar to the sleep states seen in neonates, but also that the rate of maturation of behavioural state parameter coordination *in utero* is species dependent and is intimately associated with the level of brain maturation experienced (4). The ovine fetus, a prenatal brain developer, experiences spontaneous electrical activity that can be observed in regions of the fetal cortex at ~65 days of gestation (19). As gestation progresses from this point on, there is a gradual delineation of the two distinct electrocortical activity patterns observed during their respective behavioural state, with the LV/REM and HV/NREM behavioural states being well-defined by ~ 120 days of gestation (term =145 days). The ECOG activity pattern present in ovine fetus during the LV/REM behavioural state has been characterized by electrical activity of <50 μV in amplitude and frequencies of ~13-23 Hz (20). In contrast, the ECOG activity during the HV/NREM behavioural state exhibits larger electrical wave amplitudes (>100 μV) and lower frequencies (~3-9 Hz) (20). Initially, the ovine fetus will spend a majority of its time in the LV/REM state (~50%) and ~40% of its time in the HV/NREM state. The remaining period of time will be spent in brief episodes of wakefulness or state transition.

As gestation increases towards birth, the ovine fetus will experience a progressive decrease in the incidence of the LV/REM state to ~40% by term. Consequently, there will be a progressive increase in the incidences of, primarily, wakefulness episodes and to a lesser degree, of the NREM/HV state (12). Postnatally, the ovine fetus will experience a significant decrease in the incidence of LV/REM state (<10%) and an increase in the time spent awake (12).

The ovine fetus will experience cycling of additional behavioural state-defining parameters prior to the observance of differentiated ECOG patterns, though these do not exhibit synchronization until 120 days gestation (13). In the ovine fetus, fetal ocular and nuchal muscle activities can be observed as early as ~95 days gestation (13). The occurrence of FBM, an associated physiological parameter, can be observed as early as ~40 days gestation (14). Initially, a temporal relationship between these parameters can be observed at ~105 days, with fetal EOG, nuchal muscle and breathing activity occurring together (13). As gestation advances, this relationship modifies with the developmental climax observed with at ~120 days gestation, with the presence of both ocular and fetal breathing activities coinciding with the suppression of nuchal muscle activity during the LV/REM state (13, 21).

As previously mentioned, the development of fetal behavioural states is specific to the species. The fetal behavioural state developmental pattern of the ovine fetus can be observed in other prenatal

brain developer species such as the guinea pig, baboon and monkey, all of which complete neuroanatomical maturation prior to birth (4, 22). In these species, well-defined behavioural states are present prior to birth. In contrast, postnatal brain developer species, such as the rat, cat and rabbit, which experience their most rapid rate of brain growth during the postnatal period, do not demonstrate mature behavioural state cycling until the neonatal period (23, 24).

The human fetus is classified as a perinatal brain developer, experiencing a majority of its neuroanatomical maturation during the perinatal period (4). In the human, synchronization of behavioural parameters and proper sleep state cycling occur in the later stages of fetal life at ~ 36 weeks of gestation (term = 40 weeks) (4, 10). As observed in the ovine fetus, the human fetus exhibits an early prominence for state 2F (or REMS), with its presence observed ~40% of the time (4). State 1F (or NREMS) accounts for an additional ~ 25% of total fetal time, with the remaining fetal time comprised of brief periods of wakefulness and periods of non-coincidence. As gestation progresses, the human fetus will increase the incidence of wakefulness and NREMS and decrease the amount of time in which behavioural states cannot be identified. The incidence of REMS will for the most part, remain unchanged (7). Similar to the ovine fetus, the human fetus experiences desynchronized patterns of individual behavioural parameters prior to 36 weeks of gestation, with fetal body movements evident at 8-9 weeks, FBM

at 10-12 weeks and ocular activity at 16-17 weeks of gestation (25). However, prior to 36 weeks, there is a lack of both stable temporal relationships between these parameters and a synchrony of transition periods between behavioural states (26, 27). Although there are considerable differences between species with respect to the rate of development of sleep-wake patterns and their individual parameters, all mammals appear to display a high proportion of the LV/REM state with the initial establishment of well-defined behavioural states (4).

1.1.5 Function of adult sleep state activity

For decades, scientists have attempted to determine if sleep is an active or passive process of the brain and body, with a significant amount of the investigation focusing on the results of sleep deprivation studies, which have provided evidence that sleep serves one or perhaps several life sustaining functions. In adult humans, sleep deprivation has been shown to lead to overpowering sleep pressure as well as decreased alertness and performance (28-30). Adult sleep studies in other species have shown similar evidence for sleep providing a fundamental function to life. Adult rats that experienced sleep deprivation demonstrated decreases in cerebral functioning and immune system response (31). Other studies have shown that prolonged sleep deprivation in both rats (32) and fruit flies (33) can result in death. Collectively, these results across species provide a convergent validity that suggests a necessary functional role for sleep.

Recent findings have indicated that there may be specific roles for REM and NREM sleep in the adult, given that certain brain activities tend to occur during specific times during sleep, when one of the two types of sleep is normally prevalent. The majority of these studies have used sleep deprivation to determine the function of specific sleep states. Selective sleep state deprivation studies have been shown to produce a rebound effect with respect to the state that was deprived (34).

The amount of REM sleep that an adult mammal experiences has been proposed to play an important role in the consolidation of memory. Adult rats that were selectively deprived of REM sleep were shown to perform poorer on newly learned tasks (35). This concept has been further investigated in the human adult through the use of positron emission tomography (PET) measuring regional cerebral blood flow (CBF) in specific regions of the brain that were active in subjects who learned a particular task (36). It was demonstrated that these regions were more active during the REM sleep of the trained subjects than those who were not trained in the task (36). Collectively, these results indicate a necessary function for REM sleep with respect to the consolidation of memory in the adult.

The restorative hypothesis of sleep is currently one of the most widely accepted theories explaining the function of sleep, and specifically the function of NREM sleep. The theory suggests that the decreased brain activity observed during NREM sleep promotes repair and

restoration of CNS tissues as well as the growth and synthesis of new tissues (37). Specifically, it is thought that there is a competition, as well as a need for balance, for energy in the form of adenosine triphosphate (ATP), between neural cellular energy requirements and CNS synthetic processes (37). It is therefore postulated that the lower energy requirements and decreased neuronal activity characteristic of the NREM state would allow for an increased allocation of ATP for restorative and synthetic processes. This theory is supported by PET studies demonstrating that while the brain experiences a global decrease in cerebral metabolism during NREM sleep, in comparison to waking, certain individual regions of the brain may demonstrate in increase in metabolism during NREM (38). Since NREM sleep has been shown to increase as a function of previous wakefulness and will gradually decrease in episode duration as total sleep increases (39), it has been suggested that a homeostatic regulation of the occurrence of NREM sleep exists, possibly because it carries a functional role (40).

1.1.6 Function of fetal behavioural state activity

Given the high percentage of total time the ovine fetus and human fetus spend in one of the two behavioural states, the early prominence of REM sleep activity during fetal/neonatal development, as well as the emergence of coordinated behavioural states coinciding with the period of rapid brain growth and maturation, it would be reasonable to suggest fetal and neonatal behavioural sleep states are needed for normal

neurodevelopment (41). Additionally, the distinct coordination of physiological activities that occur during behavioural state cycling, particularly with respect to FBM and cardiovascular parameters, also suggests a possible function for fetal behavioural states in proper systemic maturation (42). As in the adult, most of the behavioural state research has focused on the function of LV/REM state.

The function of LV/REM sleep in early development was initially proposed in 2 separate hypotheses (41). The first hypothesis viewed the prominence of LV/REM sleep as the result of the developing CNS being unable to inhibit a "REM sleep generator" (41). Therefore the reduction in REM sleep as development advances results from the progressive maturation of the CNS, thereby increasing its ability to suppress this generator. The second hypothesis, which tends to be more widely accepted, proposes that REM sleep provides the necessary active stimulation the CNS requires in order to develop normally, but is not able to receive from external sources since wakefulness and exposure to external stimuli is limited (41).

Selective deprivation studies have been employed in the neonate, as in the adult, to investigate the function of behavioural/sleep states, with the specific intent of investigating the role of behavioural/sleep states during development. Deprivation of REM sleep in neonatal rats with the use of pharmacological agents between postnatal days 8 to 21 was shown to be associated with increased anxiety, decreased sexual activity and

abnormal adult sleep patterns (43, 44). Anatomically, these rats also exhibited decreased brain weights and cerebral protein content (44). The role of REM sleep, like the adult, has been implicated in holding a role in learning and memory in the developing mammal. A previous study examined neonatal rat pups that were deprived of REM sleep and following the deprivation, were placed into one of two environments on postnatal Day 28: a standard control environment or an enriched environment. The exposure to these differing environments was intended to test the rats' cortical plasticity. While control rats appeared to benefit from the enriched environment, as exhibited by increased brain weights, the REM sleep deprived rats did not experience this same advantage when placed in the enriched environment. These results suggest that the absence of REM sleep during the brain development period may impair the brain's ability to respond to, or benefit from, stimulus later in life (45).

Another study explored the function of LV/REM sleep during development by investigating whether the cerebral stimulation experienced from REM sleep is unique or whether it mimicked that which was experienced during wakefulness. In this study, neonatal kittens that were also monocularly deprived would be abruptly transitioned into arousal at the onset of REM sleep. The significance of these animals also being monocularly deprived at a critical time of development lies in the finding that this condition leads to the altered development of the lateral geniculate nucleus of the thalamus (46). This developmental damage was

increased when the animal experienced REM sleep deprivation in addition to the monocular deprivation suggesting that REM sleep stimulation invokes a specific influence on the CNS that is unique in comparison to that provided by wakefulness (46). These findings collectively support the activity stimulation hypothesis of the function of REM sleep during development although it can be argued that the role of REM sleep in the fetus may differ from that of wakefulness in the neonate (47).

1.1.7 Control of behavioural/sleep state

In addition to its specific function, the regulation mechanism of sleep is also still actively being investigated. Observationally, mammalian sleep appears to be influenced by a 24-hour cycle, with the onset of sleep occurring at a regular time, given all other influences to be standard, implying that circadian regulatory processes may be involved in the regulation of sleep timing. Lesions of the suprachiasmatic nucleus (SCN) of the hypothalamus, where the circadian pacemaker is located, have been shown to produce a disturbed regulation between wakefulness and sleep which persists throughout the life of the mammal (48-50). However, these studies also demonstrated that when a SCN-lesioned animal was deprived of REM sleep, the animal still exhibited compensatory increases in REM sleep tendency, suggesting an additional and independent homeostatic influence on the control of sleep in which a propensity for sleep accumulates during wakefulness and diminishes during sleep (50).

A previous study analyzing the sleep architecture of the adult rat demonstrated a positive correlation between the duration of a REM sleep episode and that of the subsequent NREM sleep episode, suggesting that in addition to a global circadian influence on total sleep, there also existed a homeostatic sleep state cycling model for the control of adult sleep structure (51). It was proposed that the REM sleep propensity not discharged during a REM sleep episode persisted into the next new individual NREM sleep episode, with the REM sleep propensity eventually triggering the onset of the following REM episode. An additional support of this hypothesis is the finding that selective REM sleep deprivation in the adult human resulted in a significant overall increase in the number of awakenings, which may indicate an increase in REM sleep propensity given the similarity between wakefulness and REM sleep with respect EEG measurements and associated physiological parameters (52). However, waking may not be a completely functional substitute for REM sleep given that the number of awakenings increased from night to night, indicating that the high REM sleep propensity was perhaps unable to be fully dissipated (52).

Another indication of the influence of a homeostatic regulation of sleep are the results of sleep deprivation studies in which an increase in rebound sleep occurs, with both the NREM and REM sleep states being enhanced following the termination of sleep deprivation (53). An integration of a circadian influence and a homeostatic influence to

contribute to total sleep consolidation is currently the most widely accepted hypothesis on how sleep and wakefulness is regulated (54, 55).

1.2 FETAL CEREBRAL METABOLISM

1.2.1 Fetal cerebral blood flow

Studies in the ovine fetus have demonstrated an increase in CBF in the latter part of pregnancy, both with respect to cardiac output and per unit of brain weight (56). The progressive increase in CBF through fetal development is paralleled by increases in cerebral oxygen delivery, which suggests that the increased CBF is not occurring as a consequence of a drop in arterial oxygen content. Previous studies in the ovine fetus have demonstrated that there is a developmental change with respect to the regional distribution of CBF over the perinatal period, in which CBF is highest in the brain stem and reduced in the cortex prior to birth, but reverses in pattern, with CBF being greatest in the cortex and lower in the brain stem after birth (57). These developmental changes in CBF may indicate an increased importance of the brain stem structures during early development vs. later development, since the pattern of oxygen delivery also appears to transition over neuromaturation with oxygen delivery to the cortex increasing with gestational age (57). Collectively, these findings may reflect a possible regional coupling of cerebral metabolic rate (CMR) and CBF over the perinatal period (57).

1.2.2 Fetal cerebral blood flow/metabolic rate and behavioural state activity

Both fetal CBF and CMR have been shown to be influenced by behavioural state, with both shown to be increased during the LV/REM state in comparison with the HV/NREM state. An increase of ~20-25% in cerebral oxidative metabolism has been demonstrated during the LV/REM state when compared to the HV/NREM state (58, 59). Since there is evidence of a tight coupling between CBF and CMR (60), regional CBF studies have been utilized to provide insight into the CMR within specific brain regions. Previous studies in the near term ovine fetus utilizing radioactive labeled microspheres have determined an overall CBF increase of approximately 20% during the LV/REM state when compared with CBF measurements taken during the HV/NREM state, with the change being most pronounced in the midbrain and pontine structures, which are proposed to be involved in REM sleep regulation in the adult (4). The continuous measurement CBF in superior sagittal sinus of the near term ovine fetus using a transit-time flow probe produced similar results (61). The increase in CBF during the LV/REM state compared with that of HV/NREM has been shown to be evident within 1 minute of behavioural state transition. These results are similar to those performed in adult mammals in which there is a decrease in CBF during NREM sleep and a subsequent increase in CBF with the onset of REM sleep epochs (62).

While the relationship between CBF and fetal behavioural states in ovine fetus near term has been well-documented, the relationship

between behavioural state and cerebral protein synthesis is less clear. Adult studies in the rat and cat have demonstrated positive correlations between cerebral protein synthesis and REM sleep (63, 64). However, more recent studies in the adult rat and adult monkey demonstrated positive correlations between cerebral protein synthetic rate and the percentage of time spent in NREM sleep (65, 66). The significant increases in cerebral uptake of oxygen and glucose, as well as the increase in CBF during the LV/REM state compared with that of the HV/NREM state in the near term ovine fetus was initially thought to reflect an increase in neuronal functional activity and/or synthetic processes within the brain. However, the recent finding that amino acid uptake in the ovine fetal brain may instead be increased during the HV/ NREM state compared with the LV/REM state, suggests that both states have very unique and necessary roles in encouraging optimal fetal development (67).

1.2.3 Experimental techniques for measuring fetal cerebral blood flow

A number of methodologies have been employed to determine both regional and global CBF changes in the ovine fetal brain. Initially, the use of radioactive, and more recently fluorescent, microspheres has been employed to investigate regional and global changes in CBF in which each measurement represents an integrated flow over the period of time during which the microspheres are circulating (56, 68). While the microsphere technique has been shown to provide accurate measurements of CBF, the technique is limited in that it can only provide a single measurement with

respect to a specific period of time and is therefore unable to provide a continuous measurement of CBF. The ability to continuously measure behavioural state effects on CBF becomes particularly important when attempting to accurately characterize the CBF phasic stability during well-defined behavioural states and transition periods as well as during the experimental conditions.

Further attempts to continuously study CBF changes have been made by employing flow probe techniques in differing vessels in the ovine fetus, including the external carotid artery and more recently, the sagittal sinus vein. While measurements of CBF changes in the external carotid artery achieved using this methodology were shown to be positively correlated with those achieved using the radioactive microsphere technique (69, 70), this arterial blood flow is comprised of a high proportion (~60%) of extracerebral blood, which cannot be easily isolated from the cerebral circulation. Therefore caution must be employed when interpreting measurements taken from this vessel as being representative of solely CBF changes.

Measurements achieved utilizing a flow probe over the superior sagittal sinus vein in the neonatal lamb produced a strong linear correlation with arterial flow into cortical brain regions and the total brain blood flow, as determined by radioactive labeled microspheres (71). While this particular vessel's flow represented only 20% of total brain blood flow, it was comprised exclusively of cerebral blood and therefore

could be considered a reliable representative measure of CBF. Recently, this methodology has been employed in the chronically catheterized ovine fetus to measure changes in CBF in relation to behavioural state in the superior sagittal vein (61). The transit time flow probe was surgically implanted over the superior sagittal vein in the fetus. While the use of this device allowed for accurate continuous measurements of CBF in the fetus under resting conditions, as compared with measurements achieved using the microsphere technique, the surgical implantation of the transit time probe required considerable dissection, which may then affect CBF measurements. It is also possible that mechanical limitations of the flow probe may affect its ability to determine large-scale changes in CBF. This limitation of the technique may be a possible reason as to why researchers who utilized the flow probe technique to measure sagittal sinus CBF changes found only a moderate increase in CBF in relation to umbilical cord compressions, in comparison to the CBF arterial inflow measurements that were achieved using the microsphere technique (72).

1.3 SUMMARY

While the importance of sleep for the optimal biological functioning of an adult mammal has been established, the precise role and the mechanism regulating its cyclic nature have yet to be determined. Possible functions of adult sleep include providing optimal conditions for the growth and restoration of CNS tissues, the consolidation of memory and enhancement of learning. Proposed functions of adult sleep have led

to the hypothesis that adult sleep state regulation is the result of the integration between a circadian control and a homeostatic influence, with emphasis placed on the importance of proper REM sleep presence.

As in adult sleep, the precise control and function of behavioural states in the developing fetus are still unknown. It has been proposed that the behavioural state duration and cycling pattern that occurs during the period of rapid brain development is important for the proper maturation of the fetal brain and associated physiological systems. Previous studies have shown state-dependent changes in several physiological parameters such as fetal breathing, cerebral oxidative metabolism and cerebral protein synthesis. While previous studies have also found changes in CBF to be correlated with behavioural state, the need for a non-invasive technique that provides accurate and continuous measurements of CBF in the fetus near term is still present.

The higher proportion of LV/REM behavioural state at the stage of development when brain maturation is the most rapid indicates an important role for the occurrence of LV/REM. Alternatively, a recent study in the ovine fetal brain near term demonstrated that amino acid uptake was increased during the HV/NREM state compared with the LV/REM state. While the rate of incidence of both behavioural states with respect to total fetal time has been well-documented, the model of behavioural state cycling and its source of control are still unknown.

1.4 REFERENCES

1. Datta S, Maclean RR. Neurobiological mechanisms for the regulation of mammalian sleep-wake behavior: Reinterpretation of historical evidence and inclusion of contemporary cellular and molecular evidence. Neurosci Biobehav Rev. 2007;31(5):775-824.

2. Walker MP, Stickgold R. Sleep, memory, and plasticity. Annu Rev Psychol. 2006;57:139-66.

3. Rechtschaffen A, Kales A. A manual of standarized terminology, techniques and scoring system for sleep stages in human subjects. Washington, DC: US Government Printing Office; 1968.

4. Richardson BS. Ontogeny of behavioural states in the fetus. In: Thorburn GD, Harding R, editors. Textbook of fetal physiology. Oxford ; New York: Oxford University Press; 1994. p.322-328.

5. McGinty DJ, Drucker-Colin RR. Sleep mechanisms: Biology and control of REM sleep. Int Rev Neurobiol. 1982;23:391-436.

6. Curzi-Dascalova, L. and Challamel, M-J. Neurophysiological basis of sleep development. In: Loughlin GM, Carroll JL, Marcus CL, editors. Sleep and breathing in children: A developmental approach. New York: Marcel Dekker, Inc.; 2000. p.3-38.

7. Prechtl HF. The behavioural states of the newborn infant (a review). Brain Res. 1974 Aug 16;76(2):185-212.

8. Richardson BS. Metabolism of the fetal brain: Biological and pathological development. In: Hanson MA, editor. The fetal and neonatal brain stem: Developmental and clinical issues. Cambridge, England; New York: Cambridge University Press; 1991. p.87-105.

9. Nijhuis JG. Behavioural states: Concomitants, clinical implications and the assessment of the condition of the nervous system. Eur J Obstet Gynecol Reprod Biol. 1986 May;21(5-6):301-8.

10. Nijhuis JG, van de Pas M. Behavioural states and their ontogeny: Human studies. Semin Perinatol. 1992 Aug;16(4):206-10.

11. Prechtl HF. The behavioural states of the newborn infant (a review). Brain Res. 1974 Aug 16;76(2):185-212.

12. Szeto HH, Hinman DJ. Prenatal development of sleep-wake patterns in sheep. Sleep. 1985 Dec; 8(4):347-55.

13. Clewlow F, Dawes GS, Johnston BM, Walker DW. Changes in breathing, electrocortical and muscle activity in unanaesthetized fetal lambs with age. J Physiol. 1983 Aug;341:463-76.

14. Dawes GS, Fox HE, Leduc BM, Liggins GC, Richards RT. Respiratory movements and rapid eye movement sleep in the foetal lamb. J Physiol. 1972 Jan;220(1):119-43.

15. Clapp JF,3rd, Szeto HH, Abrams R, Larrow R, Mann LI. Physiologic variability and fetal electrocortical activity. Am J Obstet Gynecol. 1980 Apr 15;136(8):1045-50.

16. Zhu YS, Szeto HH. Cyclic variation in fetal heart rate and sympathetic activity. Am J Obstet Gynecol. 1987 Apr;156(4):1001-5.

17. Harding R, Sigger JN, Poore ER, Johnson P. Ingestion in fetal sheep and its relation to sleep states and breathing movements. Q J Exp Physiol. 1984 Jul;69(3):477-86.

18. Wlodek ME, Thorburn GD, Harding R. Bladder contractions and micturition in fetal sheep: Their relation to behavioural states. Am J Physiol. 1989 Dec;257(6 Pt 2):R1526-32.

19. Gluckman P, Williams CE, Gunn AJ. Brain stem and cerebral function in the fetus: Its assessment and the impact of asphyxia. In: Hanson MA, editor. The fetal and neonatal brain stem :Developmental and clinical issues. Cambridge, England ; New York: Cambridge University Press; 1991. p.289.

20. Szeto HH. Spectral edge frequency as a simple quantitative measure of the maturation of electrocortical activity. Pediatr Res. 1990 Mar;27(3):289-92.

21. Walker DW. Brain mechanisms, hypoxia and fetal breathing. J Dev Physiol. 1984 Jun;6(3):225-36.

22. Szeto HH. Behavioural states and their ontogeny: Animal studies. Semin Perinatol. 1992 Aug;16(4):211-6.

23. Jouvet-Mounier D, Astic L, Lacote D. Ontogenesis of the states of sleep in rat, cat, and guinea pig during the first postnatal month. Dev Psychobiol. 1970;2(4):216-39.

24. Shimizu A, Himwich HE. The ontogeny of sleep in kittens and young rabbits. Electroencephalogr Clin Neurophysiol. 1968 Apr;24(4):307-18.

25. Nijhuis JG, Tas BAPJ. Physiological and clinical aspects of the development of fetal behaviour. In: The fetal and neonatal brain

stem :Developmental and clinical issues. Cambridge, England ; New York: Cambridge University Press; 1991. p.268.

26. Nijhuis JG, Prechtl HF, Martin CB,Jr, Bots RS. Are there behavioural states in the human fetus? Early Hum Dev. 1982 Apr;6(2):177-95.

27. Drogtrop AP, Ubels R, Nijhuis JG. The association between fetal body movements, eye movements and heart rate patterns in pregnancies between 25 and 30 weeks of gestation. Early Hum Dev. 1990 Jun;23(1):67-73.

28. Horne J. Why we sleep :The functions of sleep in humans and other mammals. Oxford ; New York: Oxford University Press; 1988.

29. Krueger JM, Obal F,Jr, Fang J. Why we sleep: A theoretical view of sleep function. Sleep Med Rev. 1999 Jun;3(2):119-29.

30. Van Dongen HP, Maislin G, Mullington JM, Dinges DF. The cumulative cost of additional wakefulness: Dose-response effects on neurobehavioural functions and sleep physiology from chronic sleep restriction and total sleep deprivation. Sleep. 2003 Mar 15;26(2):117-26.

31. Everson CA. Functional consequences of sustained sleep deprivation in the rat. Behav Brain Res. 1995 Jul-Aug;69(1-2):43-54.

32. Rechtschaffen A. Current perspectives on the function of sleep. Perspect Biol Med. 1998 Spring;41(3):359-90.

33. Shaw PJ, Tononi G, Greenspan RJ, Robinson DF. Stress response genes protect against lethal effects of sleep deprivation in drosophila. Nature. 2002 May 16;417(6886):287-91.

34. Anch AM. Sleep :A scientific perspective. Englewood Cliffs, N.J.: Prentice Hall; 1988.

35. Karni A, Tanne D, Rubenstein BS, Askenasy JJ, Sagi D. Dependence on REM sleep of overnight improvement of a perceptual skill. Science. 1994 Jul 29;265(5172):679-82.

36. Maquet P, Laureys S, Peigneux P, Fuchs S, Petiau C, Phillips C, et al. Experience-dependent changes in cerebral activation during human REM sleep. Nat Neurosci. 2000 Aug;3(8):831-6.

37. Adam K. Sleep as a restorative process and a theory to explain why. Prog Brain Res. 1980;53:289-305.

38. Nofzinger EA, Buysse DJ, Miewald JM, Meltzer CC, Price JC, Sembrat RC, et al. Human regional cerebral glucose metabolism during non-rapid

eye movement sleep in relation to waking. Brain. 2002 May;125(Pt 5):1105-15.

39. Borbely A, Achermann P. Sleep homeostasis and models of sleep regulation. In: Kryger MH, Roth T, Dement WC, editors. Principles and practice in sleep medicine. 3rd ed. Philadelphia: W.B. Saunders; 2000. p.377-90.

40. Tononi G, Cirelli C. Sleep and synaptic homeostasis: A hypothesis. Brain Res Bull. 2003 Dec 15;62(2):143-50.

41. Roffwarg HP, Muzio JN, Dement WC. Ontogenic development of the human sleep-dream cycle. Science. 1966;152:604-19.

42. Jackson JA, Wailoo MP, Thompson JR, Petersen SA. Early physiological development of infants with intrauterine growth retardation. Arch Dis Child Fetal Neonatal Ed. 2004 Jan;89(1):F46-50.

43. Mirmiran M, van de Poll NE, Corner MA, van Oyen HG, Bour HL. Suppression of active sleep by chronic treatment with chlorimipramine during early postnatal development: Effects upon adult sleep and behavior in the rat. Brain Res. 1981 Jan 5;204(1):129-46.

44. Mirmiran M, Scholtens J, van de Poll NE, Uylings HB, van der Gugten J, Boer GJ. Effects of experimental suppression of active (REM) sleep during early development upon adult brain and behavior in the rat. Brain Res. 1983 Apr;283(2-3):277-86.

45. Mirmiran M, Uylings HB, Corner MA. Pharmacological suppression of REM sleep prior to weaning counteracts the effectiveness of subsequent environmental enrichment on cortical growth in rats. Brain Res. 1983 Mar;283(1):102-5.

46. Marks GA, Shaffery JP, Oksenberg A, Speciale SG, Roffwarg HP. A functional role for REM sleep in brain maturation. Behav Brain Res. 1995 Jul-Aug;69(1-2):1-11.

47. Mirmiran M. The function of fetal/neonatal rapid eye movement sleep. Behav Brain Res. 1995 Jul-Aug;69(1-2):13-22.

48. Mouret J, Coindet J, Debilly G, Chouvet G. Suprachiasmatic nuclei lesions in the rat: Alterations in sleep circadian rhythms. Electroencephalogr Clin Neurophysiol. 1978 Sep;45(3):402-8.

49. Edgar DM. Sleep-wake circadian rhythms and aging: Potential etiologies and relevance to age-related changes in integrated physiological systems. Neurobiol Aging. 1994 Jul-Aug;15(4):499-501.

50. Wurts SW, Edgar DM. Circadian and homeostatic control of rapid eye movement (REM) sleep: Promotion of REM tendency by the suprachiasmatic nucleus. J Neurosci. 2000 Jun 1;20(11):4300-10.

51. Benington JH, Heller HC. REM sleep timing is controlled homeostatically by accumulation of REM sleep propensity in non-REM sleep. Am J Physiol. 1994 Jun;266(6 Pt 2):R1992-2000.

52. Endo T, Roth C, Landolt HP, Werth E, Aeschbach D, Achermann P, et al. Selective REM sleep deprivation in humans: Effects on sleep and sleep EEG. Am J Physiol. 1998 Apr;274(4 Pt 2):R1186-94.

53. Greene R, Siegel J. Sleep: A functional enigma. Neuromolecular Med. 2004;5(1):59-68.

54. Borbely AA. A two process model of sleep regulation. Hum Neurobiol. 1982;1(3):195-204.

55. Dijk DJ, Lockley SW. Integration of human sleep-wake regulation and circadian rhythmicity. J Appl Physiol. 2002 Feb;92(2):852-62.

56. Rudolph AM, Heymann MA. The circulation of the fetus *in utero*. methods for studying distribution of blood flow, cardiac output and organ blood flow. Circ Res. 1967 Aug;21(2):163-84.

57. Szymonowicz W, Walker AM, Cussen L, Cannata J, Yu VY. Developmental changes in regional cerebral blood flow in fetal and newborn lambs. Am J Physiol. 1988 Jan;254(1 Pt 2):H52-8.

58. Richardson BS, Carmichael L, Homan J, Gagnon R. Cerebral oxidative metabolism in lambs during perinatal period: Relationship to electrocortical state. Am J Physiol. 1989 Nov;257(5 Pt 2):R1251-7.

59. Chao CR, Hohimer AR, Bissonnette JM. The effect of electrocortical state on cerebral carbohydrate metabolism in fetal sheep. Brain Res Dev Brain Res. 1989 Sep 1;49(1):1-5.

60. Sokoloff L. Relationships among local functional activity, energy metabolism, and blood flow in the central nervous system. Fed Proc. 1981 Jun;40(8):2311-6.

61. Czikk MJ, Totten S, Homan JH, White SE, Richardson BS. Sagittal sinus blood flow in the ovine fetus as a continuous measure of cerebral blood flow: Relationship to behavioural state activity. Brain Res Dev Brain Res. 2001 Nov 26;131(1-2):103-11.

62. Lenzi P, Zoccoli G, Walker AM, Franzini C. Cerebral blood flow regulation in REM sleep: A model for flow-metabolism coupling. Arch Ital Biol. 1999 May;137(2-3):165-79.

63. Shapiro C, Girdwood P. Protein synthesis in rat brain during sleep. Neuropharmacology. 1981 May;20(5):457-60.

64. Drucker-Colin RR, Spanis CW, Cotman CW, McGaugh JL. Changes in protein levels in perfusates of freely moving cats: Relation to behavioural state. Science. 1975 Mar 14;187(4180):963-5.

65. Ramm P, Smith CT. Rates of cerebral protein synthesis are linked to slow wave sleep in the rat. Physiol Behav. 1990 Nov;48(5):749-53.

66. Nakanishi H, Sun Y, Nakamura RK, Mori K, Ito M, Suda S, et al. Positive correlations between cerebral protein synthesis rates and deep sleep in macaca mulatta. Eur J Neurosci. 1997 Feb;9(2):271-9.

67. Czikk MJ, Sweeley JC, Homan JH, Milley JR, Richardson BS. Cerebral leucine uptake and protein synthesis in the near-term ovine fetus: Relation to fetal behavioural state. Am J Physiol Regul Integr Comp Physiol. 2003 Jan;284(1):R200-7.

68. Buckberg GD, Luck JC, Payne DB, Hoffman JI, Archie JP, Fixler DE. Some sources of error in measuring regional blood flow with radioactive microspheres. J Appl Physiol. 1971 Oct;31(4):598-604.

69. van Bel F, Roman C, Klautz RJ, Teitel DF, Rudolph AM. Relationship between brain blood flow and carotid arterial flow in the sheep fetus. Pediatr Res. 1994 Mar;35(3):329-33.

70. Gratton R, Carmichael L, Homan J, Richardson B. Carotid arterial blood flow in the ovine fetus as a continuous measure of cerebral blood flow. J Soc Gynecol Investig. 1996 Mar-Apr;3(2):60-5.

71. Grant DA, Franzini C, Wild J, Walker AM. Continuous measurement of blood flow in the superior sagittal sinus of the lamb. Am J Physiol. 1995 Aug;269(2 Pt 2):R274-9.

72. Kaneko M, White S, Homan J, Richardson B. Cerebral blood flow and metabolism in relation to electrocortical activity with severe umbilical cord occlusion in the near-term ovine fetus. Am J Obstet Gynecol. 2003 Apr;188(4):961-72.

Chapter 2

RATIONALE, HYPOTHESES AND RESEARCH OBJECTIVES

2.1 RATIONALE

In the ovine fetus, well-differentiated behavioural states are evident from 120 days gestation (term = 145 days) (1). Observations of the maturation of ECOG patterns *in utero* or of behavioural state activity from birth, indicate a similar trend in the development of sleep-wakefulness patterns in humans and other mammals, with its degree of development at birth well-correlated with the neuroanatomical development of the brain (2). A number of animal studies utilizing sleep state manipulation have indicated that the prevention of the normal occurrence of sleep state activity at certain stages of maturation in the developing mammal can lead to long-lasting anatomical and psychological effects in the individual (3-5). Collectively, these results indicate a possible functional role for behavioural state activity in developing mammal. The precise roles of the LV/ REM behavioural state and the HV/ NREM behavioural state in the developing mammal have yet to be clarified. However, recent studies have supported a stimulatory role for the LV/REM state given its association with increased CMR (1, 6) and conversely, a neuronal growth role for the HV/NREM state, as indicated by the increased rate of cerebral protein synthesis observed with this state (7).

Despite the suggested importance of normal behavioural state activity in the developing mammal, the precise cycling pattern of behavioural state activity in the ovine fetus has yet to be investigated. In the developing human, normal behavioural state cycling is indicative of a

healthy human fetus, clinically speaking, while a disrupted pattern of behavioural cycling may be indicative of CNS dysfunction, such as that characteristic of the intrauterine growth restricted fetus (1). Studies in the adult rat have demonstrated that REM sleep timing is homeostatically controlled by accumulation of REM sleep propensity in NREM sleep, such that NREM sleep epoch duration is positively correlated with prior REM sleep epoch duration, which is to be expected if the functions of REM and NREM sleep somehow interact (8, 9). Should the maturation of the developing mammal benefit from the two behavioural states in different fashions, thereby requiring adequate amounts of both states, a homeostatic control of behavioural state cycling may exist in a manner similar to that in the adult rat (8).

Previous research has suggested that the cycling between the LV/REM and HV/NREM fetal behavioural states is associated with changes in cerebral blood flow (13). A number of experimental techniques have allowed for the ability to continuously study changes in fetal CBF in ovine fetus, however all have displayed significant limitations in their use. While microspheres have been employed to investigate regional and global blood flow changes, with its validity well documented in both in the fetus and adult mammal (10, 11), one is unable to utilize this technique to measure CBF continuously. Further attempts to continuously study CBF changes were made by employing a transit-time flow probe techniques on

differing vessels in the ovine fetus, including the external carotid artery (12) and more recently the sagittal sinus (13).

The ability to continuously measure behavioural state-related changes in CBF becomes increasingly advantageous when attempting to accurately characterize changes in CBF during established behavioural states. The use of a transit-time flow probe has been utilized in a number of fetal ovine studies to continuously monitor changes in CBF as a result of both physiological and manipulated perturbations in relation to behavioural state (13, 14). The previously mentioned limitations of the existing techniques in the continuous measurement of CBF in the ovine fetus are similar to those requiring attention when studying CBF changes in the adult mammal. Recently, researchers have employed the use of a piezoelectric crystal transducer surgically placed over the sagittal sinus in the conscious adult sheep to continuously monitor cerebral blood flow velocity (CBF_v) (15). The use of the piezoelectric crystal transducer was appealing in that it allowed for the continuous measurement of changes in CBF in a conscious animal and was able to be placed directly over the vessel of interest, thereby preventing the need to disrupt the vessel by dissecting it free from surrounding tissue (15). This technique may provide a new possibility to continuously monitoring CBF in the near term ovine fetus that would be favourable compared with previously utilized methods.

2.2 HYPOTHESES

1. There will be a significant correlation between the duration of HV/NREM behavioural state epoch and that of the prior LV/REM behavioural state epoch, similar to that observed in the adult rat. This would suggest a similar homeostatic control of behavioural state cycling in the ovine fetus to that proposed for the control of sleep states in the adult rat.

2. Measurement of CBF_V in the sagittal sinus in the ovine fetus near term utilizing a piezoelectric crystal transducer will provide a continuous measure of CBF_V and reflect an increase in CBF during the LV/REM state.

2.3 OBJECTIVES

1. To examine the cycling pattern of behavioural state activity in the ovine fetus near term to determine the relationship of adjacent LV/REM and HV/NREM epoch durations in a manner similar to that in the adult rat

2. To examine the inter-epoch transition time between the LV/REM to HV/NREM states and between the HV/NREM to LV/REM states to further characterize behavioural state activity, which may have implications for control mechanisms

3. To determine the utility of a piezoelectric crystal transducer over the sagittal sinus of the near term ovine fetus to measure changes in sagittal sinus CBF_v under resting conditions in relation to changes in behavioural state.

2.4 REFERENCES

1. Richardson BS. Ontogeny of behavioural states in the fetus. In: Thorburn GD, Harding R, editors. Textbook of fetal physiology. Oxford ; New York: Oxford University Press; 1994. p.322-328.

2. Richardson B, Gagnon R. Behavioural state activity and fetal health & development. In: Creasy R., Resnik R., editors. Maternal-fetal medicine. 6th ed. Philadelphia: WB Saunders Co.; 2008 (in press).

3. Mirmiran M, van de Poll NE, Corner MA, van Oyen HG, Bour HL. Suppression of active sleep by chronic treatment with chlorimipramine during early postnatal development: Effects upon adult sleep and behavior in the rat. Brain Res. 1981 Jan 5;204(1):129-46.

4. Mirmiran M, Uylings HB, Corner MA. Pharmacological suppression of REM sleep prior to weaning counteracts the effectiveness of subsequent environmental enrichment on cortical growth in rats. Brain Res. 1983 Mar;283(1):102-5.

5. Mirmiran M, Scholtens J, van de Poll NE, Uylings HB, van der Gugten J, Boer GJ. Effects of experimental suppression of active (REM) sleep during early development upon adult brain and behavior in the rat. Brain Res. 1983 Apr;283(2-3):277-86.

6. Richardson BS, Carmichael L, Homan J, Gagnon R. Cerebral oxidative metabolism in lambs during perinatal period: Relationship to electrocortical state. Am J Physiol. 1989 Nov;257(5 Pt 2):R1251-7.

7. Czikk MJ, Sweeley JC, Homan JH, Milley JR, Richardson BS. Cerebral leucine uptake and protein synthesis in the near-term ovine fetus: Relation to fetal behavioural state. Am J Physiol Regul Integr Comp Physiol. 2003 Jan;284(1):R200-7.

8. Benington JH, Heller HC. REM sleep timing is controlled homeostatically by accumulation of REM sleep propensity in non-REM sleep. Am J Physiol. 1994 Jun;266(6 Pt 2):R1992-2000.

9. Benington JH, Heller HC. Does the function of REM sleep concern non-REM sleep or waking? Prog Neurobiol. 1994 Dec;44(5):433-49.

10. Buckberg GD, Luck JC, Payne DB, Hoffman JI, Archie JP, Fixler DE. Some sources of error in measuring regional blood flow with radioactive microspheres. J Appl Physiol. 1971 Oct;31(4):598-604.

11. Heymann MA, Payne BD, Hoffman JI, Rudolph AM. Blood flow measurements with radionuclide-labeled particles. Prog Cardiovasc Dis. 1977 Jul-Aug;20(1):55-79.

12. Gratton R, Carmichael L, Homan J, Richardson B. Carotid arterial blood flow in the ovine fetus as a continuous measure of cerebral blood flow. J Soc Gynecol Investig. 1996 Mar-Apr;3(2):60-5.

13. Czikk MJ, Totten S, Homan JH, White SE, Richardson BS. Sagittal sinus blood flow in the ovine fetus as a continuous measure of cerebral blood flow: Relationship to behavioural state activity. Brain Res Dev Brain Res. 2001 Nov 26;131(1-2):103-11.

14. Kaneko M, White S, Homan J, Richardson B. Cerebral blood flow and metabolism in relation to electrocortical activity with severe umbilical cord occlusion in the near-term ovine fetus. Am J Obstet Gynecol. 2003 Apr;188(4):961-72.

15. Upton R, Grant C, Ludbrook G. An ultrasonic doppler venous outflow method for the continuous measurement of cerebral blood flow in conscious sheep. J Cereb Blood Flow Metab. 1994 Jul;14(4):680-8.

Chapter 3

BEHAVIOURAL STATE LINKAGE IN THE OVINE FETUS NEAR TERM [1]

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3.1 INTRODUCTION

In the ovine fetus, well-differentiated electrocortical (ECOG) patterns are evident from 120 days gestation (term = 145 days) with a temporal relationship to episodic muscle activity indicative of behavioural states which are qualitatively similar to the characteristics of sleep-wake behavior in the adult (1). There is initially a high proportion of time in the low-voltage ECOG state with rapid eye movements (LV/REM) at 40-50%, with 30 to 40% time in the high-voltage ECOG state without rapid eye movements (HV/NREM) and only brief periods of wakefulness (1). Thereafter, there is a progressive decrease in the incidence of LV/REM to 30-40% of the time by term with a continued fall off in the incidence of LV/REM sleep postnatally. Observations of the maturation of ECOG patterns *in utero* or of behavioural activity from birth, indicate a similar trend in the development of sleep-wakefulness patterns in humans and other mammals, with the degree of development at birth well correlated with the neuroanatomical development of the brain, i.e., whereas sheep and primates as prenatal brain developers from a neuroanatomical standpoint have relatively mature electrocortical patterns at birth, rats as postnatal brain developers have a poorly differentiated ECOG (2).

The early prominence in LV/REM and timing across species in relation to brain growth supports a functional role in brain development. This is further supported by the finding in the ovine fetus of an increase in cerebral metabolic rate during LV/REM (1, 3, 4) presumably reflecting

increased neuronal activity with the provision of endogenous stimulation which might then promote synapse refinement and the formation of orderly connections during the 'critical period' of synaptic plasticity (5,6). Conversely, cerebral protein synthesis and degradation, i.e. turnover, appear to be increased during the HV/NREM state (7) as seen in adult animals (8,9) indicating that the decrease in the brain's metabolic rate during HV/NREM does not result from a decrease in biosynthetic activity and may, in fact, favour the synthesis of new proteins. This would support the restorative theory of sleep whereby energy conservation during NREM sleep favours the anabolic restoration of tissues (10). As such, REM and NREM behavioural state activity may both impact on the brain's development, with the former providing a degree of endogenous stimulation through neuronal activity and leading to synaptic remodeling with increased protein synthesis/degradation (11) which subsequently occurs during the following NREM period when energy needs for neuronal activity are lower.

While the precise role of sleep state activity during adult life remains elusive, there is considerable evidence that the function of REM sleep concerns some aspect of NREM sleep including a role in learning and memory consolidation through activity-dependent synaptic reorganization (12-15). Moreover, study in the adult rat has demonstrated that REM sleep timing is homeostatically controlled by accumulation of REM sleep propensity in NREM sleep, such that NREM sleep epoch

duration is positively correlated with prior REM sleep epoch duration, which is to be expected if the functions of REM and NREM sleep somehow interact (12). To our knowledge, such a relationship has not been examined for behavioural state activity earlier in life and specifically in the ovine fetus with the establishment of well-differentiated ECOG patterns which would further support a role for REM/NREM sleep state interaction in the brain's development. We have therefore examined the cycling pattern of behavioural state activity in the ovine fetus near term to determine whether the relationship of adjacent LV/REM and HV/NREM epoch durations is to that found in the adult rat (12). We have additionally examined the inter-epoch transition time from LV/REM to HV/NREM and HV/NREM to LV/REM to further characterize behavioural state activity and which may have implications for control mechanisms and their development (13, 16-18).

3.2 MATERIALS AND METHODS

3.2.1 Surgical procedures and post-operative care

Nine fetal sheep were surgically prepared at 119-128 days gestation (term = 145 days). Anesthesia was initially induced with a 40 ml injection of Pentothal into the maternal jugular vein and subsequently maintained throughout surgery with 1-1.5% halothane in oxygen (Halocarbon Laboratories, Hackensack, NJ). A polyvinyl catheter (V11, Bolab, Lake Havasu City, AZ) was placed in the maternal femoral vein prior to fetal

surgery for antibiotic (Trivetrin, Schering-Plough, Kenilworth, NJ) and fluid (1000 ml 0.9% saline solution) infusion during surgery. A midline incision was made in the maternal lower abdominal wall to expose the uterus. An incision was then made in the uterus, allowing the fetal head and upper body to be exteriorized. Polyvinyl catheters (V4, Bolab) were placed in each of the brachiocephalic arteries, a cephalic vein, and the amniotic cavity for blood sampling, and/or pressure recording, and/or antibiotic administration. Teflon-coated stainless steel wires (Cooner Wire, Chatsworth, CA) were placed biparietally on the dura for monitoring ECOG activity and through the lateral orbital ridge of the zygomatic bone of each eye for monitoring electrooccular (EOG) activity. Following the placement of the skull electrodes, the scalp was sewn over. The uterus and abdomen were closed in layers with all catheters and electrodes exteriorized to the flank of the ewe and secured to its back in a plastic pouch.

Following surgery, ewes were placed in metabolic cages suitable for continuous monitoring. Antibiotics were administered for 3 days post-operatively to the ewe via the maternal femoral vein (Trivetrin, 6ml) and to the fetus via the cephalic vein and amniotic catheters (1,000,000 IU sodium penicillin G). Animals were allowed at least 5 days of post-operative recovery, during which time maternal and fetal catheters were flushed each day with heparized saline to maintain patency and fetal arterial samples were collected for blood gas analysis. Animals were

allowed food and water *ad libitum*. All surgical, post-operative and experimental procedures followed the guidelines provided by the Canadian Council on Animal Care and the University of Western Ontario Council on Animal Care.

3.2.2 Physiological measurements

All animals were subsequently studied over an 8-hour period with continuous monitoring of behavioural parameters. Fetal ECOG and EOG activities were digitally recorded on a Powerlab® computerized data acquisition system after passing through a passive band-pass filter, 0.3 to 30 Hz on a preamplifier (model 78D, Grass Instrument Co., Oxnard CA). Fetal arterial blood samples were collected at the beginning of the study period and every three hours thereafter and analyzed for pH, PaO_2 and pCO_2 using an ABL-500 blood gas analyzer with the temperature corrected to 39.5°C (Radiometer, Copenhagen) and glucose and lactate using a YSI 2300 blood analyzer (YSI 2300 Stat Plus; Yellow Springs Instruments, Yellow Springs, OH).

Following the completion of the 8 hour study period, the ewe and fetus were immediately sacrificed. Fetal body weight was determined and the fetal brain was then rapidly removed and also weighed.

3.2.3 Data analysis

Since there were no evident changes in fetal arterial blood pH, gases, glucose and lactate levels during the course of the experiment, measurements were averaged to obtain a single value for each of these

parameters for each animal for the experimental day, with these values then averaged to obtain group means ±SEM.

The onset of behavioural state epochs was determined by visual analysis, of Powerlab® recordings (see Appendix). ECOG activity was defined to be low-voltage (LV) if the amplitude displayed was < 50 μV, high-voltage (HV) if the amplitude was 100-200 μV and intermediate-voltage (IV) if the amplitude was 50-100 μV. The criterion for a LV/REM epoch was an ECOG amplitude < 50 μV, with EOG activity present while the criterion for a HV/NREM epoch was an ECOG amplitude of 100-200 μV with the absence of EOG activity. A period of IV ECOG activity with or without the occurrence of EOG activity or a period of HV ECOG activity accompanied by the presence of EOG activity, were defined as indeterminate (ID) state activity. Eye movement activity was determined to be present if the period of EOG activity was longer than 15 seconds in duration. When a LV/REM or HV/NREM behavioural state epoch was interrupted by an IV ECOG activity period of less than 3 minute duration, the state epoch duration was calculated as the total duration of the LV or HV ECOG activity of both segments. A period of ID state activity occurring between one behavioural state epoch and the next new behavioural state epoch was termed a transition period. The beginning of a transition period was determined as the time point at which the ECOG amplitude continuously changed toward that of the next new behavioural state epoch. Accordingly, the end of a transition period was determined

as the time point at which the ECOG amplitude fulfilled the criteria for the new behavioural state epoch. LV/REM activity, HV/NREM activity and ID activity are presented as percent time of total recording time. A LV/REM-HV/NREM cycle was defined as the period inclusive of a LV/REM epoch and next new HV/NREM epoch, including the transition period between the two epochs. A HV/NREM-LV/REM cycle was defined as the period inclusive of a HV/NREM epoch and next new LV/REM epoch, including the transition period between the two epochs. Similar to the analytic definitions established in previous studies examining behavioural state activity in the ovine fetus (3,4), only behavioural state cycles with transition periods of less than a 3 minute duration and in which both behavioural state epochs within the cycle were a minimum of 3 minutes in duration were considered for analyses, which allows for the elimination of most wakefulness activity from the analyses since most wakefulness periods have been shown to be less than 3 minutes in duration (19).

Statistical analysis of data was conducted using SPSS software (SPSS 16.0 Student Graduate Version for Windows, SPSS Inc., Chicago, IL). Mean LV/REM and HV/NREM epoch duration, LV/REM-HV/NREM and HV/NREM-LV/REM cycle duration, and LV/REM to HV/NREM and HV/NREM to LV/REM transition periods were determined for each animal, which were then utilized to calculate group mean values±SEM. Comparisons of group mean durations of LV/REM and HV/NREM epochs, LV/REM-HV/NREM and HV/NREM-LV/REM cycles, and LV/REM

to HV/NREM and HV/NREM to LV/REM transition periods were determined using a paired *t*-test.

Correlation coefficients were determined for each animal using regression analysis to investigate the linkage relationship between the duration of a LV/REM ECOG epoch and that of the next new HV/NREM ECOG epoch, as well the relationship between the duration of a HV/NREM ECOG epoch and that of the next new LV/REM ECOG epoch. Group mean correlation coefficients for both linkage relationships were calculated by applying a Fisher z-transformation to the individual animal correlation coefficients, calculating the group mean z, and then applying an inverse z-transformation of the mean correlation coefficients.

To assess whether fetal maturation, oxygenation or aspects of growth impacted on the behavioural state findings for each animal, regression analyses were additionally performed determining the relationship of gestational age, PaO_2 values and fetal weight to the behavioural parameters studied. For all analyses, significance was assumed for $p<0.05$.

3.3 RESULTS

Fetal characteristic data are shown in Table 3.1 with animals between 125 and 137 days gestation at the time of study. Fetal arterial PaO_2 averaged 20.0 ±0.6 mmHg which is consistent with that previously reported for the ovine fetus near term. Fetal arterial pH, glucose and

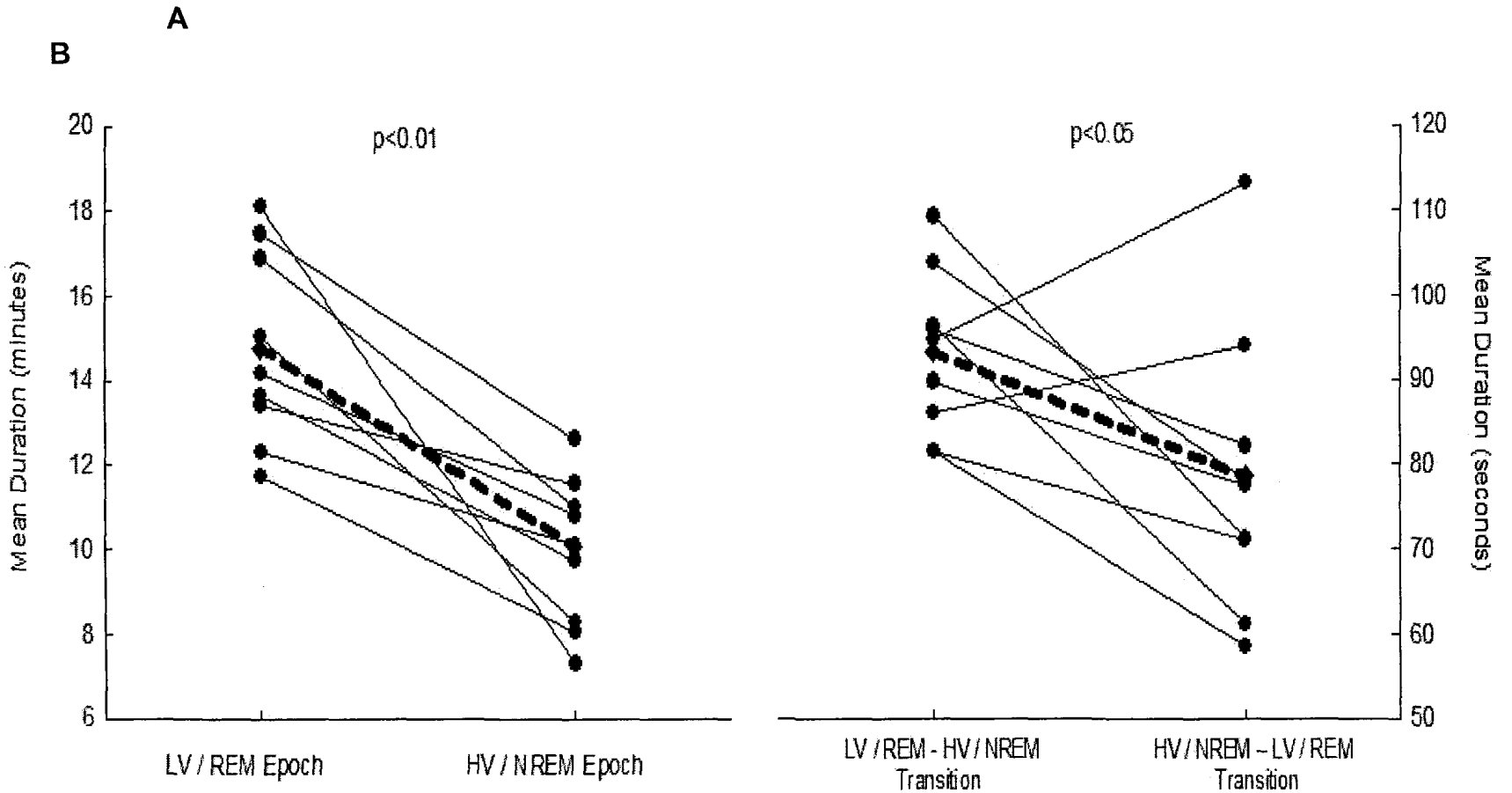
lactate concentrations averaged 7.36±0.01, 1.0±0.1 mmol/L and 1.1± 0.2 mmol/L, respectively, all of which are considered to be within the normal physiological range for the ovine fetus near term. Fetal weights ranged from 2.6 to 5.3 kg with animals somewhat heavier the more advanced the gestational age (Table 3.1).

For all animals, the mean percent time spent in LV/REM, HV/NREM and ID behavioural state activities were 52±1%, 36±1%, and 13±1%, respectively, during which electro-ocular activity was evident 94±1%, 4±1%, and 23±1% of the time for each of these behavioural state activities, respectively. The mean duration of LV/REM epochs for the nine animals was 14.8±0.8 minutes (range 12.3 to 20.3 minutes) which was significantly greater than that for HV/NREM epochs at 10.1±0.5 minutes (range 9.3 to 14.7 minutes; $p<0.01$) (Figure 3.1A). The mean duration of LV/REM-HV/NREM and HV/NREM-LV/REM cycles of behavioural state activity were 25.9±1.1 minutes, and 24.8±0.8 minutes, respectively, which were not significantly different. However, the mean duration of LV/REM to HV/NREM transition periods at 93±3 seconds (range 82 to 142 seconds) was significantly longer than that for the HV/NREM to LV/REM transition periods at 78±6 seconds (range 59 to 110 seconds) ($p<0.05$) (Figure 3.1B).

Table 3.1 Fetal sheep characteristic data presented as grouped means ± SEM; a=arterial.

Animal	Gestational Age (days)	Fetal PaO_2 (mmHg)	Fetal Weight (kg)
H103	125	20.3	2.7
50	125	19.0	2.9
919	125	19.2	3.7
H155	126	18.6	2.6
319	126	16.9	2.8
R28	133	21.4	3.4
O37	133	20.2	4.5
G46	133	20.7	5.3
Y169	137	23.7	3.2
	129 ± 1.6	**20.0 ± 0.6**	**3.5 ± 0.3**

Figure 3.1 Individual (n=9) and group mean values (dashed line) for **(A)** LV/REM and HV/NREM epoch durations and **(B)** LV/REM to HV/NREM and HV/NREM to LV/REM transition periods. Significance for group means was determined by a paired *t*-test.


A
B
p<0.01
p<0.05
Mean Duration (minutes)
Mean Duration (seconds)
20
18
16
14
12
10
8
6
120
110
100
90
80
70
60
50
LV / REM Epoch
HV / NREM Epoch
LV / REM - HV / NREM Transition
HV / NREM – LV / REM Transition

HV/NREM epoch duration was found to be positively correlated with the duration of the prior LV/REM epoch duration as shown in Figure 3.2 for the 98 LV/REM-HV/NREM cycles available for analysis. As such, LV/REM timing (the duration of the HV/NREM interval since the last LV/REM epoch) can be seen to be dependent on prior LV/REM expression which is consistent with a homeostatic control mechanism. The correlation coefficients for HV/NREM versus prior LV/REM ranged from 0.14 to 0.90 for individual animals, with a group mean correlation of 0.59 ($p<0.01$). Likewise, HV/NREM epoch duration was found to be positively correlated with the duration of the subsequent LV/REM epoch duration as shown in Figure 3.3 for the 93 HV/NREM -LV/REM cycles available for analysis. As such, LV/REM maintenance can also be seen to be dependent on prior HV/NREM epoch duration and thereby the level of accumulated LV/REM propensity at LV/REM onset. The correlation coefficients for HV/NREM versus subsequent LV/REM ranged from -0.41 to 0.78 for individual animals, with a group mean correlation coefficient of 0.46 ($p<0.01$).

Figure 3.2 HV/NREM epoch duration was found to be positively correlated with the duration of the prior LV/REM epoch duration for the 98 LV/REM-HV/NREM cycles available for analysis. A line of best fit is shown demonstrating this positive relationship.

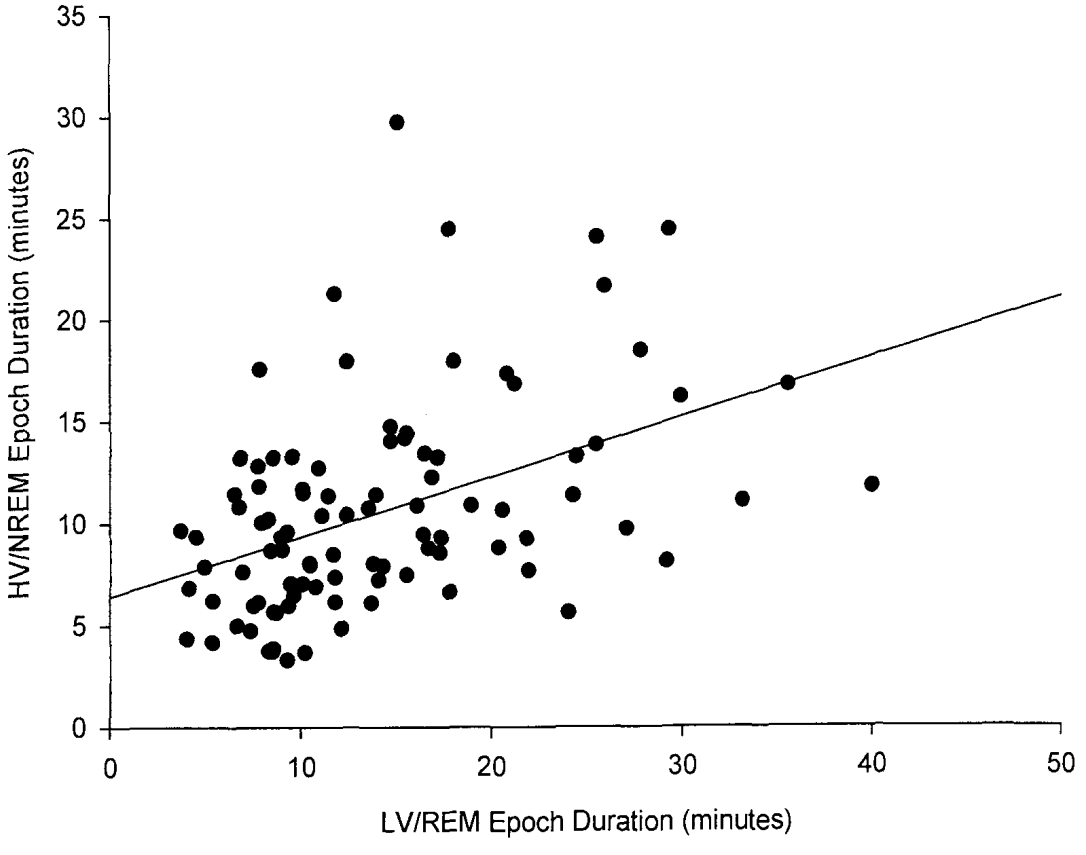
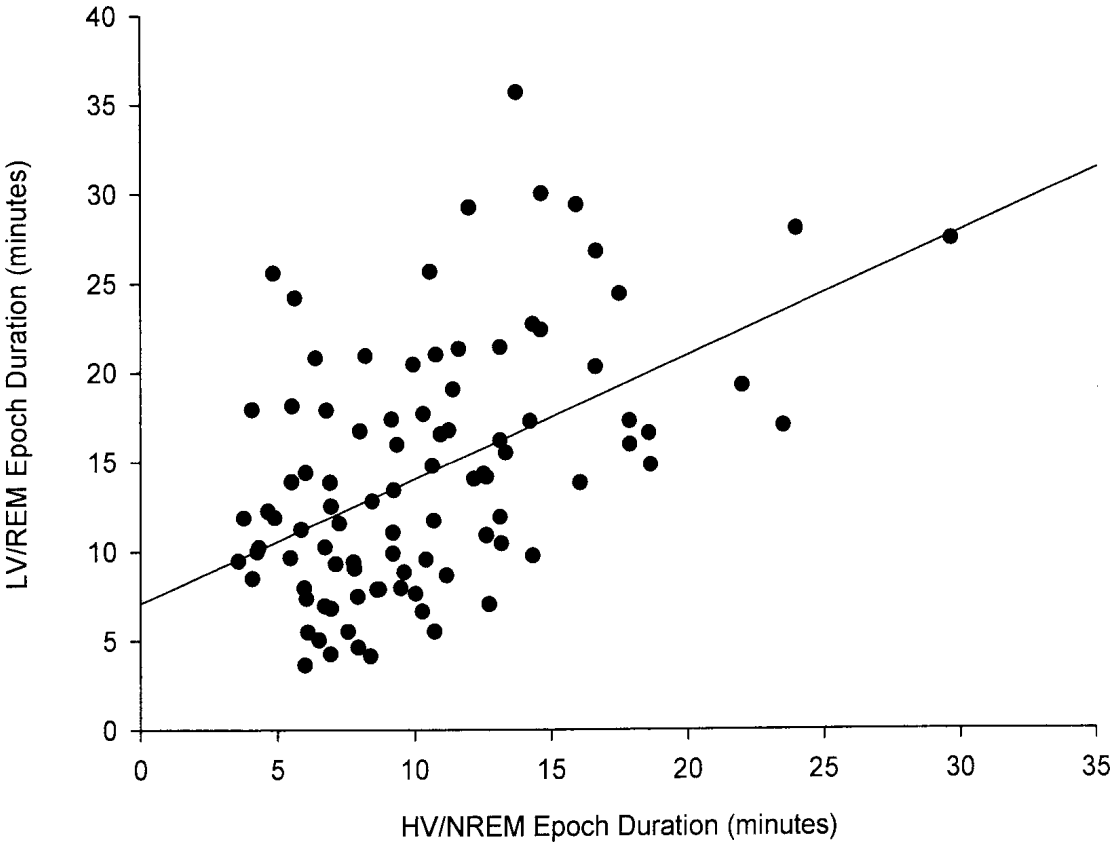

HV/NREM Epoch Duration (minutes)
35
30
25
20
15
10
5
0
0
10
20
30
40
50
LV/REM Epoch Duration (minutes)

Figure 3.3 HV/NREM epoch duration was found to be positively correlated with the duration of the subsequent LV/REM epoch duration for the 93 HV/NREM-LV/REM cycles available for analysis. A line of best fit is shown demonstrating this positive relationship.


LV/REM Epoch Duration (minutes)
HV/NREM Epoch Duration (minutes)
0
5
10
15
20
25
30
35
40

The relationship of fetal gestational age, PaO_2 values, and weight to the behavioural parameters studied was additionally assessed to determine their contribution to the range in findings across the animals. However, the only significant correlation found was that fetal weight was positively correlated with HV/NREM epoch duration ($r=0.71$, $p<0.05$).

3.4 DISCUSSION

In the present study we have further characterized behavioural state activity in the ovine fetus near term with implications for functional need and regulatory mechanisms. The LV/REM state was found to predominate as we (3) and others (19) have previously reported, and showed a mean epoch duration of ~15 minutes. This is comparable to that which we (3) and Szeto and Hinman (19) have previously reported at ~17 minutes and ~12 minutes, respectively, taking into account the slightly older animals in our earlier study and the different scoring criteria of Szeto with no 3 minute threshold requirement. The HV/NREM state showed a mean epoch duration of ~11 minutes which is likewise comparable to that which we (3) and Szeto and Hinman (19) have previously reported at ~14 minutes and ~8 minutes, respectively, after again accounting for the older animals and different scoring criteria.

HV/NREM epoch duration was found to be positively correlated with the duration of the prior LV/REM epoch duration for all animals studied and indicates that LV/REM timing or the duration of the HV/NREM

interval since the last LV/REM epoch, is dependent on prior LV/REM expression. This is consistent with a homeostatic control mechanism for REM state activity as initially proposed by Benington and Heller (12) from studies in adult rats whereby REM sleep timing is governed by accumulation of REM sleep propensity in NREM sleep which persists until it is discharged in the next REM sleep episode. It should be noted that in the present study behavioural state activity was characterized using ECOG and EOG, but not EMG criterion and identifying LV/REM activity does not differentiate that due to arousal or wakeful-like activity (19), which might then confound our findings. However, this should be minimal to negligible since wakeful-like activity in the ovine fetus at 130 days gestation is normally present only 12% of the time, occurring in short bouts lasting only 2-4 minutes (19) and should be largely excluded from the state-linkage analysis with the 3 minute threshold requirement. Furthermore, Benington and Heller (12, 13) also noted that the interval between REM sleep episodes was dependent on the total amount of NREM sleep elapsed regardless of any intervening waking which normally had little impact on the REM-NREM relationship. Of interest, the NREM state activity vs. prior REM state activity group correlation as herein determined for the near-term ovine fetus measured 0.59 whereas that reported for the adult rat measured 0.36 (12), but with considerable variance across animals for both groups and indicating that other factors must also influence NREM state duration, and thereby REM-state timing.

Fetal gestational age, PaO_2 values, and weight were not found to contribute to this variance as studied, although the one animal considered mildly hypoxic did show the weakest linkage relationship. Nonetheless, the stronger REM-NREM correlation herein noted, excepting species-specific differences in sleep state cycling and their control, may also reflect a greater functional need for the LV/REM conditions as a component of the sleep state cycle during the period of rapid growth and development for the brain and consistent with the increased incidence of REM state activity at this time (1, 2). As such, it is not surprising that drug-induced REM sleep deprivation in rat pups during the period of rapid brain maturation and when there is normally a higher amount of REM sleep, results in disturbed sleep-wake patterns during later life and a significant reduction in the size of the cerebral cortex (20).

HV/NREM epoch duration was also found to be positively correlated with the duration of the subsequent LV/REM epoch duration which differs from findings in the adult rat where REM sleep duration was largely independent of prior sleep-wake history (12). This would indicate that in the near term ovine fetus LV/REM maintenance is also dependent on prior HV/NREM epoch duration and thereby the level of accumulated LV/REM propensity at LV/REM onset, while in the adult rat factors other than the amount of REM sleep propensity to be discharged determine the duration of each REM sleep episode. While these differences may again be species-specific, they may also be developmental and reflect a greater

functional need for the HV/NREM conditions as an equally important component part of the sleep state cycle during the period of rapid growth and development for the brain and likewise consistent with the increased incidence of NREM state activity at this time (1, 19, 21, 22). As such, the positive NREM-REM correlation herein noted for the near-term ovine fetus can also be viewed in terms of increasing NREM propensity in its absence which then builds during REM state activity as the only other substantial behavioural activity at this time, until a threshold is reached thereby triggering a transition to the next NREM state epoch. Of interest then, is the developmental change whereby the increased REM state activity of early life is variably replaced by wakefulness into later life (1, 19, 21, 22), and the similarity of these two states metabolically (4, 23) and functionally (24) for the brain. Accordingly, it is possible that any NREM state-related homeostatic responses during REM state activity and seen earlier in life as herein reported, might then be replaced in later life by NREM state-related homeostatic responses now linked to wakefulness which has in fact been well established (14). Of note, the NREM state activity vs. subsequent REM state activity group correlation measured 0.46 and again showed considerable variance across the animals studied indicating that other factors likewise influence REM state duration, and thereby NREM-state timing. While fetal gestational age, PaO_2 values, and weight again did not contribute to this variance as studied, the mildly hypoxic animal was the one animal that displayed a negative linkage relationship.

State transition was examined using ECOG time-course data since this is a consistent hallmark of organized sleep state behaviour and reflects the summated activity of the neurons in the cerebral cortex and subcortex and thereby control circuitry for such activity. This analysis revealed that the mean duration of HV/NREM to LV/REM transition periods at ~78 seconds was somewhat shorter than that for the LV/REM to HV/NREM transition periods at ~93 seconds as was evident for seven of the nine animals studied. This finding of a shorter transition time when entering the LV/REM state is consistent with the abrupt increase in cerebral blood flow at transition to LV/REM versus the gradual decrease before transition to HV/NREM previously reported for the near term ovine fetus (25), to the extent that the state-related change in brain blood flow is temporally linked to the electrocorticogram. A shorter transition time when entering the 2F state which is comparable to the LV/REM state, versus the 1F state which is comparable to the HV/NREM state has also been reported for the human fetus near term (26), albeit with the limitation of using movement and heart rate activity to delineate state changes. This may involve a difference in the rate of maturation of the cycling control mechanisms for these two behavioural states with the characteristics of the 2F or LV/REM state observed earlier and with the transition time preceding this state decreasing at a faster rate than that preceding the 1F or HV/NREM state as gestation advances (1, 19, 27-29). Studies investigating the alteration between REM and NREM sleep have largely

been in adults and have led to the reciprocal interaction model proposed by McCarley and Hobson (18) whereby the transition into and out of REM sleep is produced by the reciprocal interaction between cholinergic facilitating and aminergic inhibitory neuronal populations located in the brainstem. As such, cholinergic REM-on pathways may develop earlier than aminergic REM-off pathways should McCarley and Hobson's model also be operational for behavioural state activity earlier in life, although recognizing that the temporal relation between ECOG differentiation and circuitry activation may not be precise and that governing mechanisms do not necessarily drive the organization of other sleep state phenomena.

The study of the temporal relationship between behavioural and physiological parameters has firmly established the existence of behavioural states during early life including *in utero* which are analogous to postnatal sleep states, with developmental changes whose timing is species dependent and intricately linked to brain maturation (1, 2). The early prominence of REM sleep or behavioural like activity (1, 2, 19, 21), and the increase in cerebral metabolic rate at this time (3, 4) support a role during early brain development, most likely with the provision of endogenous stimulation when waking exogenous stimulation is low which might then promote synapse refinement and the formation of orderly connections during the 'critical period' of synaptic plasticity (5, 6). The developmental change in the prominence of NREM sleep which coincides with the formation of thalamocortical and intracortical patterns of

innervation and periods of heightened synaptic remodeling (30), and the increase in protein synthesis and degradation at this time (7) likewise support a role during early brain development. Collectively, these studies also indicate that the decrease in the brain's metabolic demand during NREM sleep as seen in the ovine fetus (3, 4) and in other species postnatally, including humans (23), does not result from a decrease in biosynthetic activity and may favour the synthesis of new proteins consistent with the restorative theory of sleep (10) whereby energy conservation during NREM sleep favours the anabolic restoration of tissues. As such, REM and NREM sleep state activity may both impact on the brain's development with the former providing a degree of endogenous stimulation through neuronal activity and leading to synaptic remodeling with increased protein synthesis/degradation (11) which subsequently occurs during the following NREM period when energy needs for neuronal activity are lower. While these conjectures remain speculative, the present findings of homeostatic control for behavioural state activity in the near term ovine fetus with increasing LV/REM propensity during the HV/NREM state and increasing HV/NREM propensity during the LV/REM state further support an interaction between sleep states with the brain's development, and propensity enacting processes for these states that must be closely associated.

3.5 REFERENCES

1. Richardson BS. Ontogeny of behavioural states in the fetus. In: Thorburn GD, Harding R, editors. Textbook of fetal physiology. Oxford ; New York: Oxford University Press; 1994. p.322-328.

2. Richardson B, Gagnon R. Behavioural state activity and fetal health & development. In: Creasy R., Resnik R., editors. Maternal-fetal medicine. 6th ed. Philadelphia: WB Saunders Co.; 2008 (in press).

3. Richardson BS, Patrick JE, Abduljabbar H. Cerebral oxidative metabolism in the fetal lamb: Relationship to electrocortical state. Am J Obstet Gynecol. 1985 Oct 15;153(4):426-31.

4. Richardson BS, Carmichael L, Homan J, Gagnon R. Cerebral oxidative metabolism in lambs during perinatal period: Relationship to electrocortical state. Am J Physiol. 1989 Nov;257(5 Pt 2):R1251-7.

5. Penn AA, Shatz CJ. Brain waves and brain wiring: The role of endogenous and sensory-driven neural activity in development. Pediatr Res. 1999 Apr;45(4 Pt 1):447-58.

6. Blumberg MS, Lucas DE. A developmental and component analysis of active sleep. Dev Psychobiol. 1996 Jan;29(1):1-22.

7. Czikk MJ, Sweeley JC, Homan JH, Milley JR, Richardson BS. Cerebral leucine uptake and protein synthesis in the near-term ovine fetus: Relation to fetal behavioural state. Am J Physiol Regul Integr Comp Physiol. 2003 Jan;284(1):R200-7.

8. Nakanishi H, Sun Y, Nakamura RK, Mori K, Ito M, Suda S, et al. Positive correlations between cerebral protein synthesis rates and deep sleep in macaca mulatta. Eur J Neurosci. 1997 Feb;9(2):271-9.

9. Ramm P, Smith CT. Rates of cerebral protein synthesis are linked to slow wave sleep in the rat. Physiol Behav. 1990 Nov;48(5):749-53.

10. Adam K. Sleep as a restorative process and a theory to explain why. Prog Brain Res. 1980;53:289-305.

11. Jiang C, Schuman EM. Regulation and function of local protein synthesis in neuronal dendrites. Trends Biochem Sci. 2002 Oct;27(10):506-13.

12. Benington JH, Heller HC. REM sleep timing is controlled homeostatically by accumulation of REM sleep propensity in non-REM sleep. Am J Physiol. 1994 Jun;266(6 Pt 2):R1992-2000.

13. Benington JH, Heller HC. Does the function of REM sleep concern non-REM sleep or waking? Prog Neurobiol. 1994 Dec;44(5):433-49.

14. Benington JH. Sleep homeostasis and the function of sleep. Sleep. 2000 Nov 1;23(7):959-66.

15. Benington JH, Frank MG. Cellular and molecular connections between sleep and synaptic plasticity. Prog Neurobiol. 2003 Feb;69(2):71-101.

16. Hobson JA, Neural control of sleep, in: Turek FW and Zee PC (Eds.), Regulation of sleep and circadian rhythms. Lung biology in health disease, Vol 133, Marcel Dekker, New York, 1999, pp.81-110.

17. Merica H, Fortune RD. State transitions between wake and sleep, and within the ultradian cycle, with focus on the link to neuronal activity. Sleep Med Rev. 2004 8:473-485.

18. McCarley RW. Neurobiology of REM and NREM sleep. Sleep Med. 2007 Jun;8(4):302-30.

19. Szeto HH, Hinman DJ. Prenatal development of sleep-wake patterns in sheep. Sleep. 1985 8(4):347-355.

20. Mirmiran M, Mass YGH, Ariagno RL. Development of fetal and neonatal sleep and circadian rhythms. Sleep Med Rev. 2003 7:321-334.

21. Nijhuis JG, Prechtl HFR, Martin CB, et al. Are there behavioural states in the human fetus? Early Hum Dev. 1982 6:177-195.

22. Roffwarg HP, Muzio JN, Dement WC. Ontogenetic development of the human sleep-dream cycle. Science. 1966 152:604-619.

23. Masden PL, Vorstrup S. Cerebral blood flow and metabolism during sleep. Cerebrovasc and Brain Metab Rev. 1991 3:281-296.

24. Hobson JA, Steriade M. 1986 Neuronal basis of behavioural state control. In: Mountcastle VB, Bloom FE, Geiger SR (Eds.), handbook of Physiology, Section 1: The Nervous System, vol. IV. Intrinsic Regulatory Systems of the Brain. American Physiological Society, Bethesda, pp. 701-826.

25. Czikk MJ, Totten S, Homan JH, White SE, Richardson BS. Sagittal sinus blood flow in the ovine fetus as a continuous measure of cerebral blood flow: Relationship to behavioural state activity. Brain Res Dev Brain Res. 2001 Nov 26;131(1-2):103-111.

26. Nijhuis IJ, ten Hof J, Nijhuis JG, Mulder EJ, Narayan H, Taylor DJ, et al. Temporal organization of fetal behavior from 24-weeks gestation onwards in normal and complicated pregnancies. Dev Psychobiol. 1999 May;34(4):257-268.

27. Arduini D, Rizzo G, Massacesi M, Romanini C, Mancuso S. Longitudinal assessment of behavioural transitions in healthy human fetuses during the third trimester of pregnancy. J Perinat Med. 1991;19(1-2):67-72.

28. Groome LJ, Benanti JM, Bentz LS, Singh KP. Morphology of active sleep—quiet sleep transitions in normal human term fetuses. J Perinat Med. 1996;24(2):171-176.

29. Groome LJ, Swiber MJ, Atterbury JL, Bentz LS, Holland SB. Similarities and differences in behavioural state organization during sleep periods in the perinatal infant before and after birth. Child Dev. 1997 Feb;68(1):1-11.

30. Jacobson M. Developmental Neurobiology, New York: Plenum, 1991

Chapter 4

SAGITTAL SINUS FLOW VELOCITY IN THE OVINE FETUS AS A MEASURE OF CEREBRAL BLOOD FLOW: RELATIONSHIP TO BEHAVIOURAL STATE ACTIVITY[2]

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2 A version of this chapter has been submitted for publication: N Rao, S Hemstreet, B Matushewski, BS Richardson. *Sagittal sinus flow velocity in the ovine fetus as a measure of cerebral blood flow: Relationship to behavioural state activity.* Journal of Cerebral Blood Flow, *first submitted July 2008.*

4.1 INTRODUCTION

Interest in the biologic development of the brain and the potential for pathologic change in response to asphyxial insult has led to considerable study of the fetal cerebral circulation over the past three decades (1, 2). Much of this work has involved the use of the chronically catheterized ovine fetus and the radioactive, and more recently fluorescent, microsphere technique for blood flow determination. Although this technique has proven to be reliable and allows for regional as well as global measurements of brain blood flow, only a limited number of measurements can be made, each with limited temporal resolution. Additionally, the terminal need of tissue processing for microsphere counting limits use for other analysis. This has led to the development of continuous and less invasive measures of cerebral blood flow (CBF), including a transit time flow probe on the external carotid artery (3, 4) or the superior sagittal sinus (5), use of the coupled thermojunction technique (6), and use of laser-Doppler flowmetry (7), albeit each with limitations.

A pulsed ultrasonic Doppler venous outflow method has been developed by Upton *et al* (8) for the continuous measurement of global CBF in conscious sheep for studies of the cerebral uptake of drugs. The technique involves the placement of a 20-MHz piezoelectric crystal transducer on the superior sagittal sinus to monitor blood flow velocity and is relatively simple and inexpensive to use. Blood flow velocity

measurements have been shown to reflect actual blood flow with vessel diameter displaying little change with changes in flow velocity, as assessed from study of the velocity profile across the sinus (8). As such, this technique might also prove useful in the ovine fetus as a continuous measure of CBF which is minimally invasive and allows for longer term study.

Behavioural state activity, with similarities to postnatal sleep states, is clearly evident in the ovine fetus near term and has been shown to affect cerebral metabolism, and thereby blood flow to the brain. During the low-voltage (LV) electrocortical (ECOG) state with eye movements, the counterpart of the postnatal active sleep state or rapid eye movement (REM) sleep, both cerebral consumption of oxygen and blood flow are seen to increase by ~20% when compared to that during the high-voltage (HV) ECOG state without eye movements, the counterpart of the postnatal quiet sleep state or NREM sleep (9). In the present study, we have placed a 20-MHz piezoelectric crystal transducer on the superior sagittal sinus in the near term ovine fetus to characterize blood flow velocity measurements from this vessel and test the hypothesis that values will increase during the LV/REM state when compared to the HV/NREM state in a similar manner to that seen for CBF, i.e., by ~20%. The flow velocity profile across the sinus has also been assessed repeatedly to check the optimal sampling depth and estimated vessel diameter, and their stability both over time and with behavioural state change.

4.2 MATERIALS AND METHODS

4.2.1 Surgical procedures and post-operative care

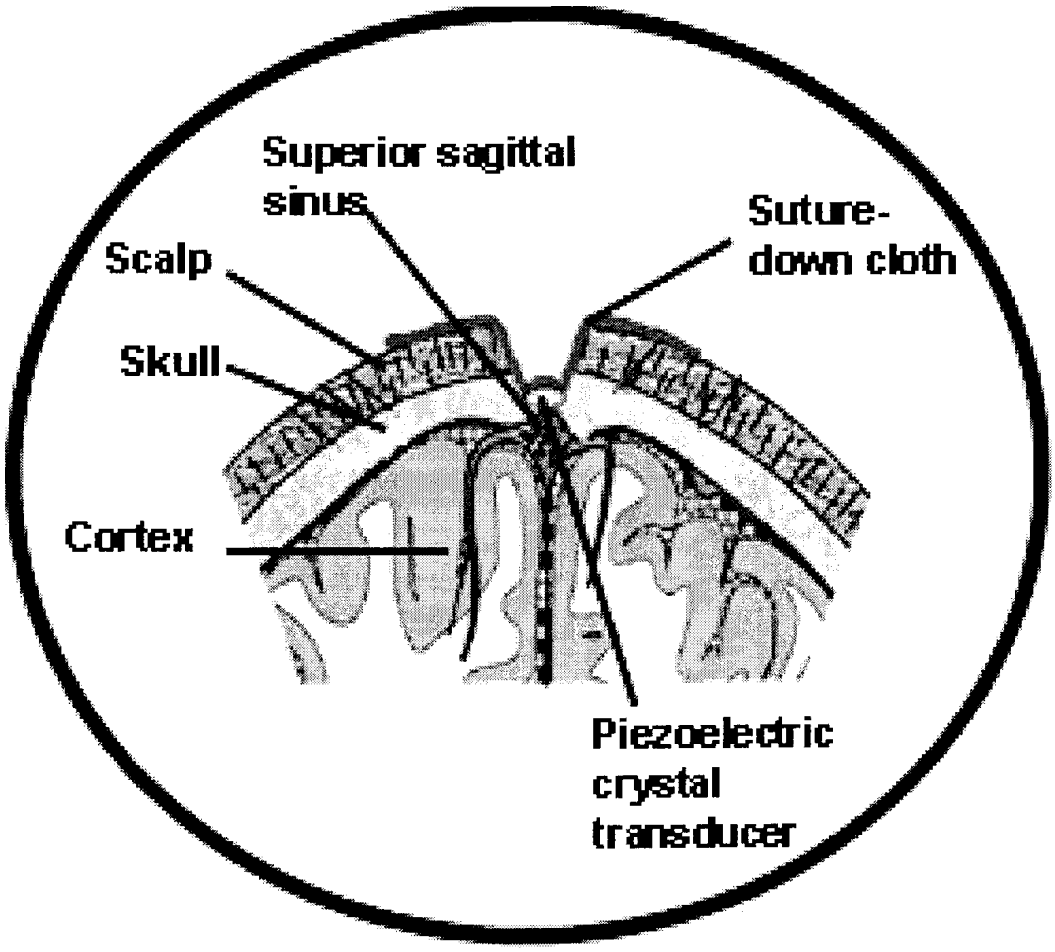
Ten sheep were surgically prepared at 119-122 days gestation (term = 145 days). Anesthesia was initially induced with a 40 ml injection of Pentothal into the maternal jugular vein and subsequently maintained throughout surgery with 1-1.5% halothane in oxygen (Halocarbon Laboratories, Hackensack, NJ). A polyvinyl catheter (V11, Bolab, Lake Havasu City, AZ) was placed in the maternal femoral vein prior to fetal surgery for antibiotic (Trivetrin, Schering-Plough, Kenilworth, NJ) and fluid (1000 ml 0.9% saline solution) infusion during surgery. A midline incision was made in the maternal lower abdominal wall to expose the uterus. An incision was then made in the uterus, allowing the fetal head and upper body to be exteriorized. For the implantation of the piezoelectric crystal transducer, a 1 cm x 0.5cm trough-like hole was created in the fetal skull along the sagittal suture just caudal to the coronal suture. A "suture-down"-style 1-mm-diameter, 20-MHz piezoelectric crystal transducer mounted on a cloth patch was placed into this opening and positioned directly over the superior sagittal sinus, resting on top of the dura matter (Figure 4.1). Prior to final fixation, the transducer's signal output was assessed at various locations along the exposed sagittal sinus to discern the location with the maximal audible signal output corresponding to the maximal velocity of blood flow in the centre of the vessel, as determined

by adjusting the sample volume depth utilizing a 545C-4 directional pulsed Doppler flowmeter (Bioengineering, University of Iowa, Iowa City, IA). The transducer was then fixed in this location using household adhesive KrazyGlue®. Teflon-coated stainless steel wires (Cooner Wire, Chatsworth, CA) were also placed biparietally on the dura for monitoring ECOG activity and through the lateral orbital ridge of the zygomatic bone of each eye for monitoring electroocular (EOG) activity. Following the placement of the piezoelectric crystal transducer and skull electrodes, the scalp flaps were approximated and sutured closed. Polyvinyl catheters (V4, Bolab) were then placed in each of the brachiocephalic arteries, a cephalic vein, and the amniotic cavity for blood sampling, and/or pressure recording, and/or antibiotic administration. The uterus and abdomen were closed in layers with all catheters, electrodes and Doppler transducer leads exteriorized to the flank of the ewe and secured to its back in a plastic pouch.

Following surgery, animals were placed in metabolic cages suitable for continuous monitoring. Antibiotics were administered for 3 days post-operatively to the ewe via the maternal femoral vein (Trivetrin, 6ml) and to the fetus via the cephalic vein and amniotic catheters (1,000,000 IU sodium penicillin G). Animals were allowed at least 3 days of post-operative recovery, during which time maternal and fetal catheters were flushed each day with heparinized saline to maintain patency and fetal arterial samples were collected for blood gas analysis. Animals were

allowed food and water *ad libitum*. All surgical, post-operative and experimental procedures followed the guidelines provided by the Canadian Council on Animal Care and the University of Western Ontario Council on Animal Care.

Figure 4.1 Schematic diagram of the surgical placement of the piezoelectric crystal transducer for CBF_v measurement


Superior sagittal sinus
Suture-down cloth
Scalp
Skull
Cortex
Piezoelectric crystal transducer

4.2.2 Physiological measurements

All animals were subsequently studied over an 8-hour period with continuous monitoring of behavioural and CBF parameters. Fetal ECOG and EOG activities were digitally recorded on a Powerlab® computerized data acquisition system (model ML795, Ad Instruments, Colorado Springs, CO) after passing through a passive band-pass filter, 0.3 to 30 Hz on a preamplifier (model 78D, Grass Instrument Co., Oxnard CA). The onset of behavioural state epochs was determined by visual analysis. The criterion for a LV/REM epoch was an ECOG amplitude < 50 μV, with EOG activity present while the criterion for a HV/NREM epoch was an ECOG amplitude of 100-200 μV and the absence of EOG activity. A period of ECOG activity with an amplitude between 50-100 μV with or without the occurrence of EOG activity or a period of HV ECOG activity accompanied by the presence of EOG activity, was defined as indeterminate (ID) state activity. A period of ID state activity occurring between one behavioural state epoch and the next new behavioural state epoch was termed a transition period.

Fetal arterial blood samples were collected at the beginning of the experimental day and every three hours thereafter and analyzed for PaO_2, pCO_2 and pH using an ABL-500 blood gas analyzer with the temperature corrected to 39.5°C (Radiometer, Copenhagen) and glucose and lactate using a YSI 2300 blood analyzer (YSI 2300 Stat Plus; Yellow Springs Instruments, Yellow Springs, OH).

Following the completion of the 8 hour study period, the ewe and fetus were immediately sacrificed. The fetal body weight was determined, with the fetal brain then rapidly removed and weighed to assess whether brain size was related to the CBF parameters measured.

4.2.3 Cerebral blood flow piezoelectric crystal transducer measurements

Equipment: The piezoelectric crystal transducer sends pulses of 20-MHz ultrasound across the sagittal sinus at an angle of approximately 45° to the vessel surface. The same transducer will receive the resulting signal echoes from the moving red blood cells, which are then amplified and processed by the Doppler flowmeter. Once received, the signal pulse is digitally recorded on the Powerlab® computerized data acquisition system and converted to a measure of velocity in millimeters per second (mm/sec) using the following equation:

$$V = \frac{\Delta f \cdot c}{2 f_o \cdot \cos\theta}$$

V = Velocity of red blood cells (mm/s)
Δf = Doppler frequency shift (kHz)
c = Velocity of sound in blood (1,565,000 mm/s)
f_o = Original / transmitted frequency from Doppler probe (20,000 kHz)
θ = Sampling angle (45°)

therefore,

$$V(mm/s) = \frac{39.125 \cdot \Delta f}{\cos 45}$$

The originating pulse is sent into the blood at time-specific intervals, resulting in the returning signal echoes being separated in time according to the distance traveled into the vessel. This mechanism allows the depth of the sampling volume within the vessel to be altered, and hence the point at which CBF velocity (CBF_V) is measured. The sampling depth or "insonation depth" can be controlled through a process referred to as "time gating" or simply "gating". The term is derived from its function of modifying the time between the transmission and reception of pulses in order to specify a sampling depth. Functionally, gating is accomplished by adjusting the "range control" of the flowmeter.

Flow Velocity Profile: The results from gating can be graphically illustrated as a flow-velocity (FV) profile, which plots the changes in velocity with respect to the changes in insonation depth over a sampling range across the vessel (Figure 4.2). Insonation depth measurements are obtained by multiplying the raw insonation depth value recorded by $\sin 45^\circ$, which corrects for the depth values obtained as a result of diagonal sampling at an angle of approximately 45° to the transducer. In the current study, gating was performed over ~60 seconds at selected time points to determine and maintain the optimal sampling depth at which the velocity signal from the flowmeter was maximal. This sampling depth, which we have termed the "peak insonation depth" (I_p), corresponds to the

intermittent maximal peak velocity determined by a FV profile as reported by Upton *et al.* (8)with study in adult sheep. On completion of each FV profile the range control on the flowmeter was readjusted as needed to maintain the optimal sampling depth at which CBF_v was then continuously recorded until the next FV profile at which time this process was repeated. From each FV profile it was also possible to estimate sagittal sinus vessel diameter by determining the minimal (I_{min}) and maximal (I_{max}) insonation depths at which positive blood flow velocity can be seen.

This was calculated using the following equation:

$$D_{ss} = \sin\theta(I_{max} - I_{min})$$

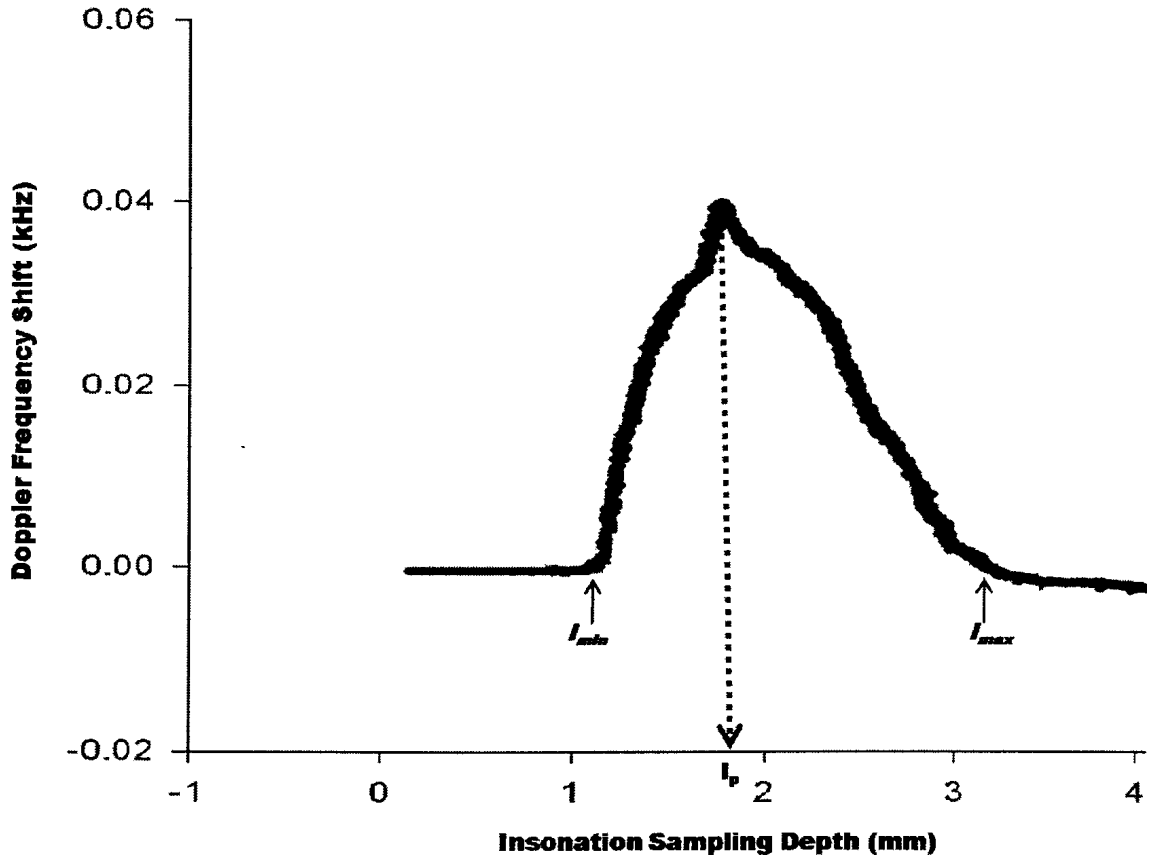
D_{ss} = Sagittal sinus vessel diameter estimate (mm)
I_{max} = Maximal sampling depth (mm)
I_{min} = Minimal sampling depth (mm)
θ = Sampling angle (45°)

CBF Velocity and Insonation Depth Measurements: CBF_v data was obtained from a 5 minute recording period during the second and third post-operative days and during the 8 hour recording period on the experimental day. Three types of CBF_v measurements were obtained: a pre-gating measurement as the integrated CBF_v recorded for the 3 minute period immediately preceding a FV profile; a post-gating measurement as the integrated CBF_v recorded for the 3 minute period immediately following a FV profile and resetting of the I_p if needed; and continous

measurements as the integrated CBF_V recorded for selected time periods without regard for FV profiling and I_p resetting. These varied measurements were obtained to investigate the stability of the I_p measurements over time and thereby of the optimal sampling depth for obtaining the maximal flow velocity. Flow velocities recorded during flow velocity profiling and I_p resetting were not included in the determination of any of the CBF_V measurements.

On the experimental day of study, a pre-gating CBF_V, post-gating CBF_V, and continuous CBF_V measurement were obtained during each of 4 LV/REM epochs and 4HV/NREM epochs for each animal. FV profiles for the pre- and post-gating measurements were carried out ~4 to 5 minutes after entry into the state epoch and only state epochs in which the pre- and post-gating measurements were each 3 minutes in duration and occurred entirely within the state epoch were included for analysis. Continuous CBF_V measurements were obtained by meaning the integrated CBF_V values recorded for the total duration of each of the behavioural state epochs examined, excepting that obtained during FV profiling and I_p resetting. A continuous CBF_V measurement was also obtained over a 5 minute period during a HV/NREM state epoch on the second and third postoperative days. The I_p, I_{min}, and I_{max} derived from each of the 4 LV/REM and 4 HV/NREM FV profilings were additionally recorded to assess the impact of behavioural state on peak insonation depth and sagittal sinus vessel diameter.

Figure 4.2 Example of velocity profile derived from gating. l_p represents the peak insonation depth, which corresponds to the peak flow velocity determined through gating; l_{min} represents the minimal insonation depth that corresponds to the first positive velocity recorded by the flowmeter; l_{max} represents the maximal insonation depth which corresponds to the last positive assess the recorded by the flowmeter.


0.06
0.04
0.02
0.00
-0.02
Doppler Frequency Shift (kHz)
-1
0
1
2
3
4
Insonation Sampling Depth (mm)

4.2.4 Data analysis

Since there were no evident changes in fetal arterial blood pH, gases, glucose and lactate levels during the course of the experiment, measurements were averaged to obtain a single value for each of these parameters for each animal for the experimental day, with these values then averaged to obtain group means ± SEM.

Pre-gating, post-gating, and continuous CBF_v measurements for the 4 LV/REM and 4 HV/NREM state epochs for each animal were averaged to obtain mean values for each of these parameters for the two behavioural states which were then used to calculate group means ± SEM. Paired *t*-test analysis was used to assess whether pre- and post- gating CBF_v, and post-gating and continuous CBF_v measurements within respective LV/REM and HV/NREM data sets differed. Paired *t*-test analysis was also used to assess whether post-gating and continuous CBF_v measurements obtained in the LV/REM state differed from corresponding HV/NREM state measurements. Repeated measures analysis of variance was used to assess whether continuous CBF_v changed during the post-operative period.

Mean I_p, I_{min}, I_{max} and D_{ss} values were likewise obtained for each animal for the two behavioural states which were then used to calculate group means ± SEM. To assess whether state-dependent changes occurred with respect to peak insonation depth and sagittal sinus vessel diameter, comparisons of group mean I_p and D_{ss} values for the LV/REM

and HV/NREM behavioural states were made using non parametric paired Wilcoxon tests and parametric paired *t*-tests, respectively. To further assess the stability of the I_p measurements and thereby of the optimal sampling depth, and of the sagittal sinus diameter measurements, their variability was also analyzed by determining the mean coefficient of variation (calculated as the I_p or D_{ss} standard deviation divided by the mean and expressed as a percentage) from the eight measurements obtained for each animal.

To assess whether fetal oxygenation or brain size impacted on the CBF parameters studied, regression analyses were additionally performed determining the relationship of PaO_2 and brain weight to continuous CBF_v and D_{ss} values meaned from the respective LV/REM and HV/NREM measurements on the experimental day of study for each animal. All statistical analysis was conducted using SPSS software (SPSS 16.0 Student Graduate Version for Windows, SPSS Inc., Chicago, IL) with significance assumed for $p<0.05$.

4.3 RESULTS

Animals ranged from 123 to 127 days gestation on the experimental day of study, with fetal arterial PaO_2 and PCO_2 values of 18.7 ± 0.4 mmHg and 49.7 ± 1.7 mmHg, respectively, which is consistent with that previously reported for the ovine fetus near term, although one animal did have a PaO_2 value at ~17mmHg which would be considered on

the low side of normal. Fetal arterial pH, glucose and lactate concentrations averaged 7.35 ± 0.01, 0.9 ± 0.1 mmol/L and 1.0 ± 0.1 mmol/L, respectively, all of which are considered to be within the normal physiological range for the ovine fetus near term. Fetal weights ranged from 2.2 to 3.0 kg with brain weights ranging from 39 to 46 grams.

The CBF_v measurement findings both within and between the LV/REM and HV/NREM behavioural states are shown in Table 4.1. Comparisons within state-related data sets revealed that post-gated CBF_v was marginally higher than pre-gated CBF_v as studied during both behavioural states by ~9 mm/sec on average although this was not significant. Post-gated CBF_v was also higher than corresponding continuous CBF_v, but this was only significant for the HV/NREM measurements, 153 ± 12 versus 138 ± 16 mm/sec, $p<.05$. Comparisons between states revealed that post-gated CBF_v and continuous CBF_v were both significantly higher during the LV/REM state than during the HV/NREM state and by ~30 mm/sec on average (both $p<.05$). Post-operative CBF_v measurements from days 2 and 3 were available for five of the animals and averaged 128 ± 17 and 135 ± 24 mm/sec, respectively, as studied over 5 minute time periods during HV/NREM, which while somewhat lower were not significantly different than corresponding CBF_v measurements on the day of experimental study.

The insonation depth and vessel diameter findings determined from the gating procedure and derived FV profiles during the LV/REM and

HV/NREM behavioural states are shown in Table 4.2. Peak insonation depth at which the velocity signal from the flowmeter was maximal was ~1.7 mm and displayed little difference as measured during the two behavioural states. Sagittal sinus vessel diameter as estimated from the minimal and maximal sampling depths at which positive blood flow velocity was seen averaged 1.7 mm and likewise was unchanged as measured during the two behavioural states. For each animal, the change in I_p and D_{ss} values across the 8 data sets analyzed were low with the mean coefficients of variation at 5.9 ± 0.8 % (range 2.8 to 9.9 %) and 7.6 ± 0.8 % (range 4.0 to 9.9 %), respectively.

The relationship of fetal PaO_2 values and brain weight to the CBF_v and D_{ss} measurements was additionally assessed to determine their contribution to the range in findings across the animals studied. However, regression analysis did not reveal any significant correlations and thereby impact from these factors on the measurement findings

Table 4.1 Cerebral blood flow velocity measurements (mm/sec).

	Pre-gated CBF_v	*p*-value	Post-gated CBF_v	*p*-value	Continuous CBF_v
LV/REM	168 ± 15	NS	177 ± 14	NS	174 ± 15
p-value	NA		*p*<.05		*p*<.05
HV/NREM	145 ± 12	NS	153 ± 12	p<0.05	138 ± 16

Data presented as grouped means ± SEM, n=10; significance determined between groups using paired *t*-test analysis; NS=non significant, NA=not applicable.

Table 4.2 Insonation depth and vessel diameter measurements (mm)

	I_p	I_{min}	I_{max}	D_{ss}
LV/REM	1.6 ± 0.1	1.1 ± 0.1	2.9 ± 0.1	1.7 ± 0.1
p-value	NS	NS	NS	NS
HV/NREM	1.7 ± 0.1	1.1 ± 0.1	2.9 ± 0.1	1.7 ± 0.1

Data presented as grouped means ± SEM, n=10; I_p=peak insonation depth, I_{min}=minimal sampling depth, I_{max}=maximal sampling depth, D_{ss}=sagittal sinus vessel diameter, NS=non significant.

4.4 DISCUSSION

We have used a piezoelectric crystal transducer on the superior sagittal sinus as a continuous measure of blood flow in the ovine fetal brain. There have been several previous studies aimed at developing a continuous measure of CBF in the ovine fetus, each with limitations to the methodology. A transit time flow probe has been placed on the external carotid artery(3, 4)and although this vessel does supply the majority of flow to the ovine brain, a significant proportion also supplies cranial structures which cannot be isolated from the cerebral circulation without extensive surgical ligation of extracerebral vessels(10, 11). The coupled thermojunction technique has also been used(6) and provides continuous, but only relative changes in CBF obtained indirectly through measurement of fluctuations in temperature from a discrete brain region. Laser-Doppler flowmetry has likewise been studied as a continuous measure of CBF (7), but again, is only able to measure relative changes in cerebral perfusion within a small region of brain tissue, which may not be linearly related to actual arterial inflow to that region. More recently, we have studied the use of a transit time flow probe on the superior sagittal sinus in the ovine fetus and while likely an accurate and quantitative measure of CBF under resting conditions(5), considerable surgical manipulation about the sinus is required including bilateral incisions through the dura which may impact on the accuracy of measurements during higher flow states(12).

The use of a piezoelectric crystal transducer on the superior sagittal sinus has been shown to reflect blood flow in adult sheep, where a strong linear correlation exists between measured flow velocity and actual flow determined using a direct venous outflow method(8). The relationship between blood velocity and blood flow would be non-linear if the vessel cross-sectional area changed or if the flow changed from laminar to turbulent at different flow rates. In their study, Upton *et al.* (8)also showed that flow remained laminar as indicated by the characteristic parabolic velocity distribution and the diameter of the sinus did not change significantly compared to the observed changes in flow velocity with the various perturbations, further supporting their velocity-to-flow findings. This is consistent with the anatomical triangular structure of the sinus, which has been reported to be fibrous in nature and not subject to alterations in size (13). However, while all animals in the Upton study showed an excellent linear relationship between velocity and flow, slopes and intercept lines varied to some extent, indicating that every animal must be individually calibrated if actual flow values, rather than change from baseline values, are required.

In the present study it was important to ensure that the optimal sampling depth for measuring CBF_V within the sagittal sinus was being maintained which was assessed by repeatedly checking the peak insonation depth and by comparing the CBF_V measurements immediately before and after each FV profile with gating adjustments as needed.

Overall, any adjustments required must have been minimal since the mean coefficient of variation for the I_p measurements was low at ~6%, and the pre- and post-gated CBF_v measurements were not significantly different. Accordingly, the post-gated and continuous CBF_v measurements during LV/REM were also similar as expected if the optimal sampling depth was being maintained and CBF throughout this time period was relatively constant. However, this was not the case for the HV/NREM measurements where post-gated CBF_v was significantly higher than continuous CBF_v which may be due to a progressive decrease in CBF during this behavioural state as suggested in our previous study with the transit-time flow probe on this vessel (5). It was also important to validate the utility of the CBF_v measurements as measures of CBF by ensuring that the cross sectional area of the sinus was being maintained which was assessed by repeatedly checking the sinus diameter both over time and with the state-related change in flow-velocity. To the extent that flow-related changes in cross sectional area will similarly affect the sinus diameter then this must also be minimal since the mean coefficient of variation for D_{ss} was again low at ~8%, and this measurement was exactly the same at 1.7 mm for the two behavioural states. This value here reported for the near term ovine fetus is somewhat less than that reported for adult sheep at ~2.5mm (14) which was also noted by Upton *et al* (8), and is consistent with the expected increase in sinus size with increasing growth of the brain and the associated increase in blood flow through this

vessel from ~18 ml/min in the near term ovine fetus (5) to ~40 ml/min in adult sheep (8).

With the optimal sampling depth and the sinus diameter minimally changed as herein studied under resting conditions, then CBF_v should accurately reflect corresponding blood flow in the superior sagittal sinus thereby providing a continuous measure of CBF in the ovine fetus. While we have not confirmed and calibrated this relationship by checking CBF_v values against a known 'gold standard' for CBF measurement, it is reassuring that post-gated and continuous CBF_v increased ~20% on average during the LV/REM state when compared to the HV/NREM state which is similar to that we (9) and others (15, 16) have reported for CBF using the microsphere technique. Of interest, a cross-sectional area for the superior sagittal sinus in the near term ovine fetus can be estimated if one assumes that this vessel is roughly triangular in shape with a depth of ~1.7mm and a dural base width of ~2 mm, which is reasonable since 1 mm OD catheters are often chronically placed in this vessel (5, 9). Then a CBF_v of 156 mm/sec as seen on average in the present study would equate to a blood flow of ~16 ml/min which is remarkably similar to that we have previously reported for this vessel at ~18 ml/min as measured with a transit-time flow probe (5) and further supports the utility of this technique for measurement of CBF in the ovine fetus.

In the newborn lamb, the measured flow in the superior sagittal sinus is mainly from frontal cortex and superior regions of the anterior parietal

cortex and represents ~20% of total brain blood flow (17). Although representing venous outflow from the brain, changes in arterial inflow are promptly reflected in outflow changes and the use of a flow probe on this vessel has validated the flow measurements as an accurate and quantitative measure of CBF since a strong linear correlation exists between sinus flow and total brain blood flow as measured using the microsphere technique (17). We have also demonstrated the utility of a transit-time flow probe on the superior sagittal sinus in the ovine fetus as a continuous measure of CBF under resting conditions (5). However, this technique requires considerable surgical manipulation about the sinus which may impact on the accuracy of measurements during higher flow states (12), and highlights the existence of alternative venous outflow tracts as a well known anatomic feature (18). We now demonstrate the utility of using a 20-MHz piezoelectric crystal transducer on the superior sagittal sinus to monitor blood flow velocity as a measure of CBF in the ovine fetus. Placement of the crystal transducer is minimally invasive, taking no more than 15 minutes at the time of animal surgery, with signal processing/data collection relatively simple and inexpensive. The optimal sampling volume and sinus diameter showed little variance as studied under resting conditions, consistent with that reported in adult sheep (8), and flow-velocity values were as expected both in relation to behavioural state change and to actual blood flow measurements previously made from this vessel (5, 9, 15, 16, 19). As such, CBF_v should accurately reflect

corresponding blood flow in the superior sagittal sinus and thereby provide a continuous measure of CBF in the ovine fetus, to the extent that this venous outflow remains similarly linked to arterial inflow within the brain. However, it should again be noted that CBF_v measurements are at best a relative measure of CBF unless a calibration process is undertaken, and may not accurately reflect CBF under other study conditions, for example with hypoxia and increased blood flow giving rise to vessel distortion and/or turbulence, and in the preterm fetus where a smaller sagittal sinus may impair the ability to maintain the optimal sampling depth. Accordingly, the assessment of optimal sampling depth and vessel diameter along with CBF_v should be an integral part of the measurement process when using this technology under all study conditions.

4.5 REFERNECES

1. Richardson BS. Metabolism of the fetal brain: Biological and pathological development. In: Hanson MA, editor. The fetal and neonatal brain stem :Developmental and clinical issues. Cambridge, England; New York: Cambridge University Press; 1991. p.87-105

2. Richardson BS. The fetal brain: Metabolic and circulatory responses to asphyxia. Clin Invest Med. 1993 Apr;16(2):103-14.

3. van Bel F, Roman C, Klautz RJ, Teitel DF, Rudolph AM. Relationship between brain blood flow and carotid arterial flow in the sheep fetus. Pediatr Res. 1994 Mar;35(3):329-33.

4. Gratton R, Carmichael L, Homan J, Richardson B. Carotid arterial blood flow in the ovine fetus as a continuous measure of cerebral blood flow. J Soc Gynecol Investig. 1996 Mar-Apr;3(2):60-5.

5. Czikk MJ, Totten S, Homan JH, White SE, Richardson BS. Sagittal sinus blood flow in the ovine fetus as a continuous measure of cerebral blood flow: Relationship to behavioural state activity. Brain Res Dev Brain Res. 2001 Nov 26;131(1-2):103-11.

6. Abrams RM, Gerhardt KJ, Burchfield DJ. Behavioural state transition and local cerebral blood flow in fetal sheep. J Dev Physiol. 1991 May;15(5):283-8.

7. Lan J, Hunter CJ, Murata T, Power GG. Adaptation of laser-doppler flowmetry to measure cerebral blood flow in the fetal sheep. J Appl Physiol. 2000 Sep;89(3):1065-71.

8. Upton R, Grant C, Ludbrook G. An ultrasonic doppler venous outflow method for the continuous measurement of cerebral blood flow in conscious sheep. J Cereb Blood Flow Metab. 1994 Jul;14(4):680-8.

9. Richardson BS, Patrick JE, Abduljabbar H. Cerebral oxidative metabolism in the fetal lamb: Relationship to electrocortical state. Am J Obstet Gynecol. 1985 Oct 15;153(4):426-31.

10. Baldwin BA, Bell FR. The anatomy of the cerebral circulation of the sheep and ox. the dynamic distribution of the blood supplied by the carotid and vertebral arteries to cranial regions. J Anat. 1963 Apr;97:203-15.

11. Dunnihoo DR, Quilligan EJ. Carotid blood flow distribution in the *in utero* sheep fetus. Am J Obstet Gynecol. 1973 Jul 1;116(5):648-56.

12. Kaneko M, White S, Homan J, Richardson B. Cerebral blood flow and metabolism in relation to electrocortical activity with severe umbilical cord occlusion in the near-term ovine fetus. Am J Obstet Gynecol. 2003 Apr;188(4):961-72.

13. Capra NF, Kapp JP. Anatomic and physiologic aspects of venous system. In: Wood JA, editor. Cerebral blood flow: Physiologic and clinical aspects. New York: McGraw-Hill; 1987. p.37-58.

14. Petrov YY, Prough DS, Deyo DJ, Klasing M, Motamedi M, Esenaliev RO. Optoacoustic, noninvasive, real-time, continuous monitoring of cerebral blood oxygenation: An in vivo study in sheep. Anesthesiology. 2005 Jan;102(1):69-75.

15. Rankin JH, Landauer M, Tian Q, Phernetton TM. Ovine fetal electrocortical activity and regional cerebral blood flow. J Dev Physiol. 1987 Dec;9(6):537-42.

16. Chao CR, Hohimer AR, Bissonnette JM. The effect of electrocortical state on cerebral carbohydrate metabolism in fetal sheep. Brain Res Dev Brain Res. 1989 Sep 1;49(1):1-5.

17. Grant DA, Franzini C, Wild J, Walker AM. Continuous measurement of blood flow in the superior sagittal sinus of the lamb. Am J Physiol. 1995 Aug;269(2 Pt 2):R274-9.

18. Hegedus, SA, Shackelford RT. A comparative-anatomical study of the cranio-cervical venous systems in mammals, with special reference to the dog: Relationship of anatomy to measurements of cerebral blood flow. Am J Anat. 1965 Mar;116:375-86.

Chapter 5

GENERAL DISCUSSION, FUTURE STUDIES AND CONCLUSIONS

5.1 GENERAL DISCUSSION

The maturation of ECOG patterns in humans and other mammals have been shown to be well-correlated with the neuroanatomical development of the brain, suggesting that behavioural state activity may provide a functional role in brain development (1). The early prominence of the LV/REM state in developing mammals has been suggested to reflect increased neuronal activity that, in turn, may promote synaptic modulation (2, 3). Conversely, recent research conducted in the near term ovine fetus has demonstrated that ^{13}C-leucine amino acid uptake was increased during the HV/NREM state (19), suggesting that the incidence of the HV/NREM state may promote the synthesis of new proteins. As such, if both the LV/REM and HV/NREM behavioural states influence brain maturation, it is possible that the two states may interact in order to ensure optimal development. The purpose of the current project was 2-fold. The first aim of the project was to examine the relationship of adjacent LV/REM and HV/NREM epoch durations in order to determine the cycling pattern of behavioural state activity in the ovine fetus near term and gain insight into the interaction between the two behavioural states. The second aim of the project was to characterize the changes in CBF_v in the superior sagittal sinus of the near term ovine fetus utilizing a 20-Mhz piezoelectric crystal transducer and determine whether the CBF_v changes observed in relation to behavioural state resembled the changes demonstrated for CBF under the same conditions.

As in the fetus, the precise roles of sleep state activity in the adult remain elusive. However, there is considerable evidence that the two sleep states may interact to influence each others' functions. For example, it has been suggested that REM sleep relates to certain aspects of NREM sleep including a role in learning and memory consolidation through activity-dependent synaptic reorganization (5-8). The examination of sleep state cycling in the adult rat demonstrated that NREM sleep epoch duration is positively correlated with the prior REM sleep epoch duration, suggesting that REM-state timing may be homeostatically controlled by accumulation of REM sleep propensity in NREM sleep, thereby implying that the functions of REM and NREM sleep somehow interact (6). Such a relationship had yet to be investigated with respect to behavioural state activity during the period of neurodevelopment in the ovine fetus. The current study demonstrated that there is an interactive relationship between the LV/REM and HV/NREM epoch durations, which differs slightly from the interactive sleep state relationship demonstrated in the adult rat. Similar to the results of sleep architecture studies in the adult rat (6), the current study found a positive correlation existing between the HV/NREM state epoch duration and the duration of the prior LV/REM epoch. This finding suggests that the duration of the HV/NREM epoch duration is dependent on prior LV/REM expression and therefore the accumulation of LV/REM state propensity accumulated in the HV/NREM state will persist until discharged in the next LV/REM epoch. The current

study also demonstrated a positive correlation between the duration of the HV/NREM epoch and the duration of the subsequent LV/REM epoch, a finding that was not demonstrated with the adult rat. This would indicate that in the near term ovine fetus, LV/REM maintenance is additionally dependent on the level of accumulated LV/REM propensity at LV/REM onset. This finding may also reflect a propensity for the HV/NREM state that accumulates during the LV/REM state until a threshold is reached, thereby triggering the onset of the next HV/NREM state epoch. This developmental uniqueness in the interactive relationship between the two behavioural states, compared with the adult, may reflect a greater functional need by the fetus for the HV/NREM conditions by the fetus during this period of rapid brain growth and development. Collectively, these results suggest a homeostatic control mechanism for behavioural state cycling for the near term ovine fetus whereby there exists an association between the increasing LV/REM propensity during the HV/NREM state and the increasing HV/NREM propensity during the LV/REM. That, in turn, facilitates an interactive relationship between the two behavioural states to ensure optimal brain development.

In Chapter 4, work was presented in which a 20-Mhz piezoelectric crystal transducer crystal was utilized to continuously measure changes in CBF_V in superior sagittal sinus of the near term ovine fetus. Much of the brain blood flow determination in studies utilizing the chronically catheterized near term ovine fetal model had been accomplished utilizing

the microsphere technique, and more recently, the transit time flow probe. While the microsphere technique allows for accurate CBF measurements, the technique allows for only a limited number of measurements to be made. Additionally, the terminal tissue preparation required for microsphere counting limits the use of the tissue for other analysis. The use of the transit-time flow probe to measure superior sagittal sinus blood flow has been shown to produce continuous accurate measurements of CBF, though a considerable amount of surgical manipulation is required for the placement of the device, which may impact on the accuracy of high flow states (4, 9). The use of a 20-MHz piezoelectric crystal transducer on the sagittal sinus to continuously monitor CBF_v has been shown to be effective in studying global changes in CBF in the adult sheep (10). CBF_v measurements in the adult sheep have been shown to reflect changes in actual blood flow with the vessel diameter demonstrating little modifying with changes in flow velocity, as assessed from study of the velocity profile across the sinus (10). This minimally invasive technique has yet to be used in the fetus and would provide an additional option for the continuous measurement of CBF changes in sagittal sinus of the ovine fetus.

In the present study we were able to demonstrate the utility of using a 20-MHz piezoelectric crystal transducer on the superior sagittal sinus to continuously monitor CBF_v as a measure of CBF in the ovine fetus. Measurements derived from use of this technique demonstrated flow-

velocity values as expected both in relation to behavioural state change and to actual blood flow measurements previously made from this vessel (1, 4, 11-13). Studies of CBF in the sagittal sinus of the newborn lamb have demonstrated that changes in arterial inflow are promptly reflected in venous outflow changes and that the CBF measurements derived from the use of a flow probe on this vessel are accurate and quantitative measures of CBF, since a strong linear correlation exists between sinus flow and total brain blood flow, as determined by the microsphere technique (14). Therefore, the CBF_v measurements achieved using the piezoelectric crystal transducer technique should accurately reflect corresponding changes in blood flow in the superior sagittal sinus and thereby provide a continuous measure of CBF in the ovine fetus. Using a FV profile, we were able to determine that the optimal sampling depth and sinus diameter experienced minimal variance as studied under resting conditions, which was consistent with that reported in the adult sheep (10). Collectively, these results suggest the 20-Mhz piezoelectric crystal transducer is able to provide continuous accurate CBF_v measurements under resting conditions, when the optimal sampling depth and vessel diameter are minimally altered. Since this technique has yet to be validated by a calibration process in the ovine fetus, it is best to consider CBF_v measurements as a relative measure of CBF in the ovine fetal brain. Previous studies have demonstrated that under hypoxic conditions, the transit-time flow probe produced reduced CBF measurements in

comparison to those achieved utilizing the microsphere technique (9), suggesting that sagittal sinus vessel may experience alternations under large scale changes in flow that an externally applied CBF measuring device may not be able to accommodate. Given that the piezoelectric crystal transducer has yet to be tested under such experimental perturbations or in animals where the sagittal sinus vessel is anatomically smaller, such as the case in preterm animals, it is suggested that the optimal sampling depth and vessel diameter be assessed along with CBF_v regularly during the course of a study.

5.2 FUTURE STUDIES

The extent to which the fetus will compensate for the reduced incidence of a particular behavioural state has yet to be clarified. The manipulation of behavioural/sleep states in adult studies and fetal studies have been accomplished using a number of methodologies, including the introduction of pharmacological agents known to alter sleep architecture. Previous studies in the ovine fetus have utilized the mixed nicotinic and muscarinic cholinergic agonist carbachol to increase the incidence of LV ECOG activity and eye movements and the muscarinic cholinergic antagonist scopolamine to increase the presence of HV ECOG activity (15). While these agents were shown to be capable of manipulating behavioural state in the ovine fetus, there appeared to be no "rebound compensation" of the deprived state in the recovery period following the

termination of the agent infusion (15) which is in contrast to similar studies performed in the adult mammal (16). The manipulation of behavioural state in this study, however, was conducted only for a period of 90 minutes. In a more recent study, behavioural state manipulation in the near term ovine fetus was achieved by systemically infusing the adenosine A_1-receptor agonist cyclopentyladenosine (CPA), which nearly completely abolished the occurrence of the LV/REM state, and the experimental adenosine A_{2A} receptor antagonist ZN-241385 to increase the incidence of LV ECOG activity and eye movements for a period of 1 hour (20). It would worthwhile to replicate these studies with a longer infusion period in order to determine the extent, if any, to which the fetus will attempt to compensate for the reduced incidence of the deprived state. In order to assess the influence of normal behavioural state cycling in the ovine fetus, comparisons of various brain regions from normally cycling animals vs. those from animals that experienced disrupted cycling can be made. In the human fetus, it has been shown that the intrauterine growth restricted fetus has a delayed development of behavioural states and displays disrupted cyclic alternation between states, which may be associated with a relatively immature CNS (17). Additionally, previous studies have suggested that while the LV/REM state may provide endogenous stimulation to the developing mammal that may be necessary for optimal synaptic neuromodulation (2, 18), the rate of protein synthesis has been shown to be increased during the HV/NREM state in the ovine

fetus (19). These results, in addition to those of the current study, would suggest that the significant deprivation of either behavioural state would prevent the developing brain from optimally maturing. Thus, the extended selective behavioural state deprivation in the near term ovine fetus and its subsequent effect on neurodevelopment would provide increased insight into the interrelationship between the two behavioural states.

While the utility of a 20-MHz piezoelectric crystal transducer on the superior sagittal sinus in the near term ovine fetus has been shown to determine changes in CBF_V continuously, a calibration study, similar to that performed by Grant *et al.* (14) which assessed the accuracy of the transit-time flow probe to continuously measure CBF, would be worthwhile to perform with the piezoelectric crystal transducer. This calibration study would determine the extent to which the CBF_V measurements achieved utilizing the piezoelectric crystal transducer are representative of changes in CBF. Additionally, an assessment of the piezoelectric crystal transducer to detect large scale changes in CBF would determine its utility in fetal perturbation studies. In a recent study examining CBF changes during umbilical cord occlusions, the change in CBF detected by the transit-time flow probe was noticeably lower than that determined using the microsphere technique, suggesting that the flow probe technique on the sagittal sinus may be unable to achieve accurate measurements during high flow rate changes (9). It is possible that under such conditions, the vessel diameter of the sagittal sinus changes significantly, leading to a

change in optimal sampling depth, which would alter the accuracy of the CBF_v measurements when utilizing the piezoelectric crystal transducer. Similarly, the utility of the technique may be different if there is a significant anatomical alteration of the sagittal sinus vessel, such as smaller sinus vessel diameter that may be found in a preterm fetus. Assessing the utility of the technique in such an animal model would help determine the extent to which vessel diameter may be distorted without significantly altering the accuracy of the measurements.

5.3 CONCLUSIONS

In conclusion, this thesis was focused on determining the relationship between adjacent LV/REM and HV/NREM epoch durations, inter-epoch transition period durations and the possible control models governing behavioural state cycling as well as associated changes in CBF_v as it relates to behavioural state. The major findings of this research project were:

1. HV/NREM epoch duration was found to be positively correlated with the durations of the prior LV/REM epoch, which was similar in findings to those determined adult rat studies, and also with the durations of the subsequent LV/REM epoch, which was not found in studies with the adult rat.

2. The mean duration of LV/REM to HV/NREM transition periods was significantly longer than that for the HV/NREM to LV/REM transition periods.

3. Behavioural state dependent changes in superior sagittal sinus blood flow velocity determined in the near term ovine fetus utilizing a 20-MHz piezoelectric crystal transducer, demonstrated increased CBF_V during the LV/REM state in comparison with the HV/NREM state and displayed minimal variation in optimal sampling depth and vessel diameter under resting conditions.

These studies have provided a basis for future work centering on the effects of alternation of behavioural state cycling on state-influenced physiological parameters such as CBF and protein synthesis as well as the fetal requirement for proper behavioural state cycling. Furthermore, the studies presented herein provide a better understanding of the possible control mechanisms of fetal behavioural state cycling during periods of increased brain maturation and growth.

5.4 REFERENCES

1. Richardson BS. Metabolism of the fetal brain: Biological and pathological development. In: Hanson MA, editor. The fetal and neonatal brain stem :Developmental and clinical issues. Cambridge, England; New York: Cambridge University Press; 1991. p.87-105.

2. Penn AA, Shatz CJ. Brain waves and brain wiring: The role of endogenous and sensory-driven neural activity in development. Pediatr Res. 1999 Apr;45(4 Pt 1):447-58.

3. Blumberg MS, Lucas DE. A developmental and component analysis of active sleep. Dev Psychobiol. 1996 Jan;29(1):1-22.

4. Czikk MJ, Totten S, Homan JH, White SE, Richardson BS. Sagittal sinus blood flow in the ovine fetus as a continuous measure of cerebral blood flow: Relationship to behavioural state activity. Brain Res Dev Brain Res. 2001 Nov 26;131(1-2):103-11.

5. Benington JH, Heller HC. Does the function of REM sleep concern non-REM sleep or waking? Prog Neurobiol. 1994 Dec;44(5):433-49.

6. Benington JH, Heller HC. REM sleep timing is controlled homeostatically by accumulation of REM sleep propensity in non-REM sleep. Am J Physiol. 1994 Jun;266(6 Pt 2):R1992-2000.

7. Benington JH. Sleep homeostasis and the function of sleep. Sleep. 2000 Nov 1;23(7):959-66.

8. Benington JH, Frank MG. Cellular and molecular connections between sleep and synaptic plasticity. Prog Neurobiol. 2003 Feb;69(2):71-101.

9. Kaneko M, White S, Homan J, Richardson B. Cerebral blood flow and metabolism in relation to electrocortical activity with severe umbilical cord occlusion in the near-term ovine fetus. Am J Obstet Gynecol. 2003 Apr;188(4):961-72.

10. Upton R, Grant C, Ludbrook G. An ultrasonic doppler venous outflow method for the continuous measurement of cerebral blood flow in conscious sheep. J Cereb Blood Flow Metab. 1994 Jul;14(4):680-8.

11. Richardson BS, Patrick JE, Abduljabbar H. Cerebral oxidative metabolism in the fetal lamb: Relationship to electrocortical state. Am J Obstet Gynecol. 1985 Oct 15;153(4):426-31.

12. Rankin JH, Landauer M, Tian Q, Phernetton TM. Ovine fetal electrocortical activity and regional cerebral blood flow. J Dev Physiol. 1987 Dec;9(6):537-42.

13. Chao CR, Hohimer AR, Bissonnette JM. The effect of electrocortical state on cerebral carbohydrate metabolism in fetal sheep. Brain Res Dev Brain Res. 1989 Sep 1;49(1):1-5.

14. Grant DA, Franzini C, Wild J, Walker AM. Continuous measurement of blood flow in the superior sagittal sinus of the lamb. Am J Physiol. 1995 Aug;269(2 Pt 2):R274-9.

15. Morrison JL, Carmichael L, Homan J, Richardson BS. The effects of 'sleep promoting agents' on behavioural state in the ovine fetus. Brain Res Dev Brain Res. 1997 Oct 20;103(1):1-8.

16. Antonioli M, Solano L, Torre A, Violani C, Costa M, Bertini M. Independence of REM density from other REM sleep parameters before and after REM deprivation. Sleep. 1981;4(2):221-5.

17. Richardson BS. Ontogeny of behavioural states in the fetus. In: Thorburn GD, Harding R, editors. Textbook of fetal physiology. Oxford ; New York: Oxford University Press; 1994. p.322-328.

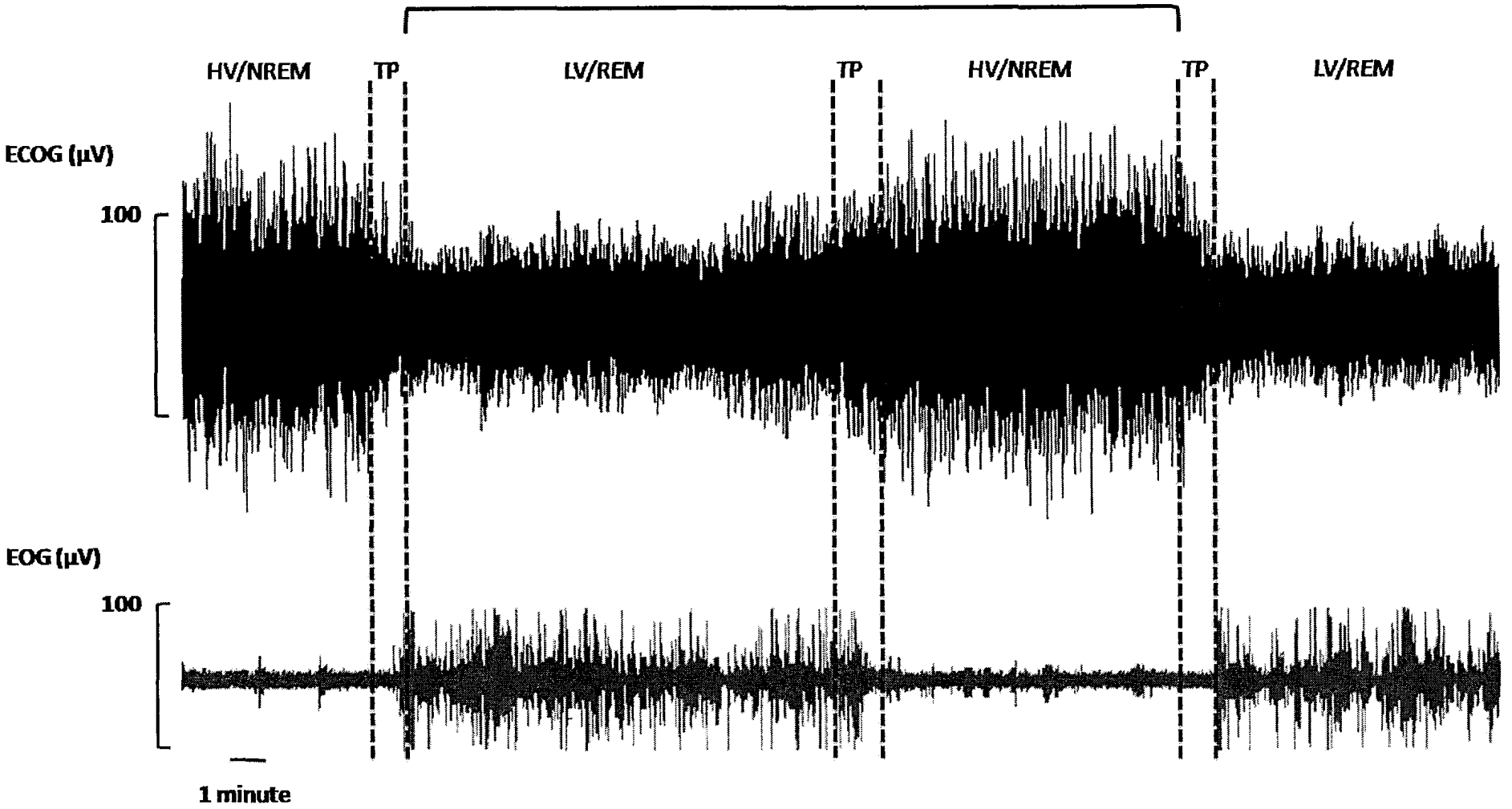
18. Mirmiran M. The function of fetal/neonatal rapid eye movement sleep. Behav Brain Res. 1995 Jul-Aug;69(1-2):13-22.

19. Czikk MJ, Sweeley JC, Homan JH, Milley JR, Richardson BS. Cerebral leucine uptake and protein synthesis in the near-term ovine fetus: Relation to fetal behavioural state. Am J Physiol Regul Integr Comp Physiol. 2003 Jan;284(1):R200-7.

20. Koos BJ, Maeda T and Jan C. Adenosine A(1) and A(2A) receptors modulate sleep state and breathing in fetal sheep. J.Appl.Physiol. 2001.91(1): 343-350

APPENDIX

A.1 - Chart recording of a sheep fetus at 125 days gestation, demonstrating ECOG and EOG activities. State transition periods (TP) are denoted by the hatched lines and were determined by visual analysis of progressive change in ECOG amplitude. The bracket denotes one complete LV/REM – HV/NREM cycle.


HV/NREM
TP
LV/REM
TP
HV/NREM
TP
LV/REM
ECOG (μV)
100
EOG (μV)
100
1 minute