Neurodevelopmental effects of placental restriction in sheep

by

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Abstract

Intrauterine growth restriction (IUGR) and poor perinatal growth in humans are associated with poorer cognition and memory and altered functional lateralisation. Altered brain morphology and neurodevelopment following IUGR appears responsible, and may be ameliorated by neonatal catch-up growth, however assessing relative effects of prenatal and postnatal growth on cognition in humans is difficult due to environmental confounders. Experimental placental restriction (PR) in sheep, via surgical removal of uterine epithelial attachment sites prior to mating, restricts intrauterine growth and is followed by catch-up growth. Cognitive consequences have not been examined in this model. Effects of sex, age and prior learning on cognition were therefore characterised in control (CON) sheep, then effects of PR on learning, memory, cognition, functional and morphological lateralisation were investigated.

Size at birth and neonatal fractional growth rates during the first 16 days of life (ie. neonatal catch-up growth) were measured for CON and PR offspring. Behavioural testing occurred at 18 and 40 weeks old. In maze tasks, trials and time per task, bleats and arm entries were recorded for initial learning (L), memory (M1, M2) and reversal (R1, R2) tasks. Behavioural lateralisation was recorded using obstacle avoidance and maze exit preference tasks, and structural lateralisation were measured in the prefrontal cortex brain region at 52 weeks of age.

In CON sheep, naive sheep aged 18 or 40 weeks required longer to complete task R1 than 40 week olds retested after learning the task at 18 weeks old, indicating prior learning was recalled at later ages. The exit route used for earlier learning tasks also predicted speed required to solve task R1 in 40N females.

Body weight and skull size at birth did not differ between CON and PR lambs utilised for behavioural testing. At 18 weeks, placentally restricted male lambs took more trials to solve the initial learning task, but required less time to complete task R1 than control males. Trials and time required to solve task M1 in 40 week old males correlated negatively with neonatal growth. Bleat frequency during task R1 in 18 week old females correlated positively with birth weight and neonatal fractional growth rate.

In 40 week old females, PR were more strongly lateralised in the maze exit preference task lateralisation than CON. Lateralisation direction was consistent between ages in PR females only, and was more consistent between tasks at 18 weeks in PR than CON females. Behavioural lateralisation did not correlate with perinatal growth, and brain morphology at 52 weeks did not differ between treatments. Correlations between perinatal growth and adult brain morphology were largely limited to males, whereas correlations between behaviour and brain morphology existed largely in females.

In conclusion, effects of age, sex and experience on cognitive and behavioural outcomes must be taken into account when evaluating these outcomes in sheep. Effects of PR on cognition and behavioural lateralisation were limited but suggested sex-specific programming of postnatal neurodevelopment. Neonatal growth rate correlated with memory performance in males, suggesting interventions during this period may improve outcomes.

Statement of Originality

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Damien S. Hunter

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Abbreviation list

18N – 18 week-old sheep naive to cognitive and behavioural tests

40E – 40 week olds retested after learning the task at 18 weeks old

40N - 40 week-old sheep naive to cognitive and behavioural tests

AGA – born at a birth weight appropriate for gestational age

ACTH – adrenocorticotrophic hormone

APIB - Assessment of Preterm Infant Behaviour

BSID - Bayley Scale of Infant Development

BW – birth weight

CA1-4 – cornu ammonis fields 1-4

CC - corpus callosum

CON – controls

CX – carunclectomy model

DG – dentate gyrus

DTI – diffusion tensor imaging

EQ – energy quotient (energy intake/kg body weight per day)

FA – functional anisotropy

FGR – fractional growth rate

fMRI – functional magnetic resonance imaging

GA – gestational age

GW – gestational week

HPA – hypothalamic-pituitary-adrenal axis

IQ – intelligence quotient

IUGR – intrauterine growth restriction

LBW – low birth weight

MRI – magnetic resonance imaging

NBAS - Neonatal Behavioural Assessment Scale

PR – placentally restricted

PVH - paraventricular hypothalamic nucleus

SES – socioeconomic status

SGA – born at a birth weight small for gestational age

Task L – initial learning task

Task M1 – first memory task

Task R1 – first reversal task

Task M2 – second memory task

Task R2 – second reversal task

THROM - thromboxane A_2 analogue (STA_2) administration

UN – maternal gestational undernutrition

UPE - uteroplacental vessel bed embolisation

UPL - uteroplacental vessel ligation

VMH - ventromedial hypothalamic nucleus

Outputs arising from thesis

Publications directly arising from thesis

1. **Hunter, D. S.**, Hazel, S.J., Kind, K.L., Liu, H., Marini, D., Owens, J.A., Pitcher, J.B., Gatford, K.L. (2015). "Do I turn left or right? Effects of sex, age, experience and exit route on maze test performance in sheep." Physiology and Behavior 139: 244–253.

2. **Hunter, D. S.**, Hazel, S.J., Kind, K.L., Liu, H., Marini, D. Giles, L.C., De Blasio, M. J., Owens, J.A., Pitcher, J.B., Gatford, K.L. (2015). "Placental and fetal growth restriction, size at birth and neonatal growth alter cognitive function and behaviour in sheep in an age- and sex-specific manner." Physiology and Behavior 152(Pt A): 1-10.

3. **Hunter, D.S.**, Hazel, S.J., Kind, K.L., Owens, J.A., Pitcher, J.B., Gatford, K.L. (2016). "Programming the brain: Common outcomes and gaps in knowledge from animal studies of IUGR." Physiology & Behavior 164, Part A: 233-248.

4. **Hunter, D. S.**, Hazel, S.J., Kind, K.L., Liu, H., Marini, D. Giles, L.C., De Blasio, M. J., Owens, J.A., Pitcher, J.B., Gatford, K.L. (2016). "Effects of induced placental and fetal growth restriction, size at birth and early neonatal growth on behavioural and brain structural lateralization in sheep." Laterality (Accepted paper, 28/9/16)

Collaborative publications related to this thesis

5. Wooldridge, A. L., Bischof, R.J., Meeusen, E.N., Liu, H., Heinemann, G.K., **Hunter, D.S.**, Giles, L.C., Kind, K.L., Owens, J.A., Clifton, V.L., Gatford, K.L. (2014). "Placental restriction of fetal growth reduces cutaneous responses to antigen after sensitization in sheep." American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 306(7): R441-446.

6. Liu, H., Schultz, C.G., De Blasio, M.J., Peura, A.M., Heinemann, G.K., Harryanto, H., **Hunter, D.S.**, Wooldridge, A.L., Kind, K.L., Giles, L.C., Simmons, R.A., Owens, J.A., Gatford, K.L. (2015). "Effect of placental restriction and neonatal exendin-4 treatment on postnatal growth, adult body composition, and in vivo glucose metabolism in the sheep." American Journal of Physiology - Endocrinology and Metabolism 309(6): E589-E600.

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7. **D Hunter**, H Liu, KL Gatford, JA Owens, KL Kind, J Pitcher, S Hazel 2012 Maternal dietary methyl supplementation normalises brain structure in the placentally-restricted sheep. *26th National Workshop on Fetal and Neonatal Physiology, Port Stephens, Australia, March 2012*

8. **D Hunter**, KL Gatford, KL Kind, H Liu, M De Blasio, JA Owens, J Pitcher, S Hazel 2013 Placental restriction of fetal growth induces sex-specific changes to learning in maze tasks in adolescent and young adult sheep. *27th National Workshop on Fetal and Neonatal Physiology, Barossa Valley, Australia, April 2013*

9. **DS Hunter**, S Hazel, KL Kind, H Liu, D Marini, L Giles, JA Owens, J Pitcher1, KL Gatford 2014 Low birth-weight and poor postnatal growth correlate with poorer memory and cognitive flexibility in male IUGR sheep in maze tasks. *27th National Workshop on Fetal and Neonatal Physiology, Yanchep, Australia, April 2014*