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High oxytocin infants gain more mass with no additional maternal energetic costs in wild grey seals (*Halichoerus grypus*)

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ABSTRACT

Maximising infant survival requires secure attachments and appropriate behaviours between parents and offspring. Oxytocin is vital for parent-offspring bonding and behaviour. It also modulates energetic balance and neural pathways regulating feeding. However, to date the connections between these two areas of the hormone's functionality are poorly defined. We demonstrate that grey seal (*Halichoerus grypus*) mothers with high oxytocin levels produce pups with high oxytocin levels throughout lactation, and show for the first time a link between endogenous infant oxytocin levels and rates of mass gain prior to weaning. High oxytocin infants gained mass at a greater rate without additional energetic cost to their mothers. Increased mass gain in infants was not due to increased nursing, and there was no link between maternal mass loss rates and plasma oxytocin concentrations. Increased mass gain rates within high oxytocin infants may be due to changes in individual behaviour and energy expenditure or oxytocin impacting on tissue formation. Infancy is a crucial time for growth and development, and our findings connect the oxytocin driven mechanisms for parent-infant bonding with the energetics underlying parental care. Our study demonstrates that oxytocin release may connect optimal parental or social environments with direct physiological advantages for individual development.

1. Introduction

Parental attachment and care giving behaviours are of fundamental importance to reproductive success in many species. Throughout the mammalian clade, maternal bonding and nurturing behaviours are of particular importance, and infant survival is frequently solely dependent on how mothers interact with their offspring. Mothers cannot succeed in raising offspring without some degree of co-ordination between parties to accomplish the common goal of infant survival to independence (Fleming et al., 1999). Cognitive and physiological systems that promote behavioural synchrony across parent-infant dyads play a vital role in this co-ordination. However, any mechanism that enables parent-infant interactions must function despite changing infant cognitive abilities as they develop across the period they are dependent on their parent(s) (Rice and Barone, 2000). Therefore, in infants, physiological systems mediating behavioural expression may be key to keeping dependent offspring with their parents and ensuring infants act appropriately towards them and other conspecifics.

The neuropeptide hormone oxytocin (OT) is vital for both social and parental bonding, plays a key role in the initiation of maternal behaviour and in some species mediates the continuance of good quality infant care throughout the dependent period (Gimpl and Fahrenholz, 2001; Ross and Young, 2009; Rilling and Young, 2014). At birth, a mother's OT release initiates bonding with her infant and maternal care (Gimpl and Fahrenholz, 2001; Ross and Young, 2009). It has been theorised that OT then acts in a positive feedback loop within mother-infant pairs to develop secure attachment between the two and to mediate maternal behaviour directed towards the infant (Rilling and Young, 2014; Nagasawa et al., 2012). A mother's OT feedback loop is initiated via filial infant stimuli causing additional OT release in the mother after birth (Strathearn et al., 2009). This OT expression has been shown to trigger care giving behaviours towards human infants while activating dopamine 'reward' systems a mother's brain (Strathearn et al., 2009), and in humans there is high co-expression between OT and dopaminergic receptor genes to facilitate this (Quintana et al., 2019). Then, by performing care giving behaviours towards her infant, a mother is more likely to be exposed to additional infant stimuli that causes even more OT release in the mother, perpetuating the 'loop' and generating elevated OT concentrations within securely attached mothers (Rilling and Young, 2014). This positive

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feedback loop is also theorised to exist in the infant, with good quality maternal care causing infant attachment to the mother and OT release due to parental stimuli (Kojima et al., 2012), generating high OT concentrations in the infant. Therefore, if double positive OT feedback loops exist in mother-infant pairs, with one loop in each individual, high OT mothers should also have high OT infants (Rilling and Young, 2014). Experiments using non-filial socially bonded individuals show that positive OT feedback loops exist across individuals in social contexts (Nagasawa et al., 2015). However, there is no evidence to date that such loops exist within mother-infant pairs, due to a lack of data on infant OT responses alongside their mother's OT concentrations.

While the effects of changing OT concentrations within mothers is well studied (Gimpl and Fahrenholz, 2001), impacts on infants, or the physiology of peripheral tissues, remain poorly understood. There is evidence from laboratory manipulation studies that OT influences the development of a variety of peripheral tissues (Uvnäs-Moberg et al., 1998; Elabd et al., 2014; Colaianni et al., 2015; Rault et al., 2015) and exposure to OT during infancy can have long term impacts on weight gain (Uvnäs-Moberg et al., 1998), as this time period is crucial for body growth and formation (Metcalfe and Monaghan, 2001). In humans (*Homo sapiens*) problems with infant nutrition and development are estimated to cause 45% of deaths in children under five years old globally, with suboptimal breastfeeding, growth stunting and wasting critically affecting child development and survival in the first 1000 days of life (Black et al., 2013). Current interventions to overcome infant 'failure to thrive' in humans, such as complimentary feeding, only show modest success in tackling these problems (Dewey and Adu-Afarwuah, 2008) and understanding physiological mechanisms driving an infant's ability to gain weight and mature is therefore of great importance. If the mass changes induced via OT manipulations in laboratory settings can be detected in natural systems, then elevation of infant OT through successful bonding and interacting with maternal figures would be a fundamental driver of an infant's ability to thrive and reach independence.

Grey seals (*Halichoerus grypus*) are colonially breeding marine mammals, with females that produce one pup per year. The pups are nursed on high fat milk while mothers fast before weaning abruptly approximately 18 days post-partum (Pomeroy et al., 1999). They present an excellent model system to study maternal behaviour and physiology as blood samples can be collected from both adults and infants, mothers are solely responsible for raising pups to independence, are individually identifiable and the entire dependent period can be observed in a relatively short time period for a large mammal. Additionally, of the few OT systems studied in animal species in the wild, to date the most is known about grey seals (Robinson et al., 2014; 2015; 2017). In this study mother-pup pairs were monitored to assess whether mothers with high OT concentrations produced pups with high OT concentrations, and whether the variation in OT concentrations within mothers and pups were correlated to patterns of mass change across the dependent period.

2. Materials and methods

2.1. Study sites and animals

Field work was conducted on the island of North Rona (NR), Scotland (59∘06′N, 05∘50′W) and the Isle of May (IoM), Scotland (56∘11′N, 02∘33′W), both grey seal breeding colonies with long term research projects. Data and samples were collected from both colonies during the winter breeding season in 2010 and 2011. Across the two study years, plasma samples were collected from 66 mothers and their pups (36 from NR, 30 from the IoM). 20 mothers occurred in both study years (11 from NR, 9 from the IoM). Mothers were identified by unique markings (natural pelage patterns, or applied tags or brands (Smout et al., 2011)). Sampling was restricted to mothers first seen either prepartum or with newborn pups. We attempted to capture mother-pup pairs twice during the lactation period to obtain plasma samples at 1–7 days after the pup's birth ('early lactation') then 9–15 days after the first sampling event ('late lactation') (Robinson et al., 2015a). We also attempted to re-capture as many pups post-weaning as possible during the natural 1–4 week post-weaning fast in this species (Reilly, 1991), and sampled 43 weaned study pups (15 from NR, 28 from the IoM).

2.2. Mass measurements, plasma and milk sampling and analysis

Grey seal mothers with pups were approached, captured, weighed and sampled as previously described (Pomeroy et al., 1999; Robinson et al., 2015a). The use of chemical immobilization ameliorates physiological stress responses to capture and handling in phocid seals (Harcourt et al., 2010), and prior validation studies have shown that in grey seals, there was no change in plasma OT with handling time (Robinson et al., 2014, 2015b) and no difference in extracted plasma OT levels across chemically immobilized or physically restrained seals (Robinson et al., 2014). Plasma samples were collected by venipuncture, transported to a field laboratory and stored frozen at −20 °C as described in Robinson et al. (2014; 2015). Our capture protocol meant that there was always a 10-minute wait for mothers to become immobilised before a plasma sample could be collected. This wait would eliminate any plasma OT peaks triggered by pre-capture nursing as OT has a short half-life in plasma (Robinson et al., 2014). It is typically only possible to obtain milk samples from seal mothers after an intravenous OT injection, however this could have confounded endogenous OT concentrations in the milk collected. Using plastic 20 ml syringes adapted for drawing milk, two milk samples were successfully collected from grey seal mothers without the use of exogenous OT. The analysis protocol for milk samples supplied with the OT ELISA (see above) was followed with two alterations, detailed in the supplementary materials (Appendix A. Methods), to prevent the high fat content of the milk (60%, (Iverson et al., 1993)) interfering with the assay.

Plasma was analysed for OT in duplicate using an ELISA (produced by Assay Designs Inc. at the time of this analysis, ELISA kit is currently produced by Enzo Life Sciences but uses a different antibody) with each sample undergoing solid-phase extraction prior to analysis following methodology previously validated for detecting phocid plasma OT (Robinson et al., 2014). Plates were read using a BioTek ELx800 reader. The standard curve and assay results for all plates were fitted using the calibFit package (Haaland et al., 2011) in R version 2.15.0 (R Development Core Team, 2012). Recovery rates for the extraction and ELISA procedure were 107.2% (n = 10), inter-assay coefficient of variance (COV) over the 14 plates used in this study was 16.1% and intra-assay COV for this assay was 3.5%.

2.3. Statistical analysis

All analyses were performed using the statistical package R 3.4.1 (R Development Core Team, 2012).

Plasma concentrations for mothers and their pups in early and late lactation were compared using a one-way ANOVA. The data were analysed after a natural log transformation as the original data were not normally distributed (Shapiro Wilk test, $p < 0.001$). Basal plasma OT concentrations were also calculated for the 43 post-weaning pups that we were able to locate on the colony. The OT concentrations from these individuals during early lactation (with mother), late lactation (with mother) and post-weaning (without mother) were compared using a one-way ANOVA. The data were analysed after a natural log transformation as the original data were not normally distributed (Shapiro Wilk test, $p < 0.001$).

GAMMs (Wood, 2006) were used to analyse variables affecting the OT concentration detected in dependent pups and for exploring the relationships between variables affecting mass gain in pups and mass loss in mothers. Details of model construction, selection process and the final model coding are given in the supplementary materials (Appendix

A. Methods), For the GAMMs investigating pup mass gain and mother mass loss, rates of mass change were calculated in kg/day for all mother-pup pairs which had mass measurements and were sampled for plasma OT detection in both early and late lactation (n = 58 mother-pup pairs). Larger grey seal mothers lose mass at a faster rate than smaller mothers (Iverson et al., 1993); therefore, the rate of mass loss (kg/day) for all mothers was transformed by dividing mass loss rates by the mother's mass at first capture, during early lactation. This gave individual mass specific rates of mass loss for all mothers for use in subsequent analysis. In pups, plasma OT concentrations detected in early and late lactation were significantly positively correlated ($r = 0.54$, $p < 0.001$, 95% CIs [0.32, 0.7], Appendix A. Methods, Fig. A1) and therefore a mean of the two values was used to correlate with mass gain. Mother plasma OT concentrations across the early and late sampling points were not significantly correlated ($r = 0.12$, $p = 0.37$, 95% CIs [−0.14, 0.37], Appendix A. Methods, Fig. A2) and therefore concentrations from early and late lactation were analysed separately with the transformed mass loss rate.

3. Results

3.1. OT concentrations in mothers and pups

Basal plasma OT concentrations in pup plasma were significantly higher than those detected in mothers throughout early and late lactation (Fig. 1, ANOVA: $F_{3,232} = 141.4$, $p < 0.001$). No significant differences were detected between pups in early and late lactation (mean ± SE: 21.9 ± 1.5 pg/ml and 19.9 ± 1.4 pg/ml respectively, Tukey honest significant difference test, $p = 0.5$) or mothers in early and late lactation (mean ± SE: 8.2 ± 0.6 pg/ml and 7.6 ± 0.5 pg/ml respectively, Tukey honest significant difference test, $p = 0.7$). Maternal plasma OT concentrations ranged from 3.5 to 25.5 pg/ml in early lactation and 3.5–16.9 pg/ml in late lactation. Pup plasma OT concentrations ranged from 11.5 to 48.1 pg/ml in early lactation and 8–52.2 pg/ml in late lactation. There was a significant positive relationship between pup plasma OT concentration and that of its mother (Fig. 2, GAMM: $R2 = 0.34$, $p = 0.02$, Appendix B. Table B.1). Pups from NR also had significantly higher plasma OT concentrations than pups from the IoM (Fig. 2, $p < 0.001$).

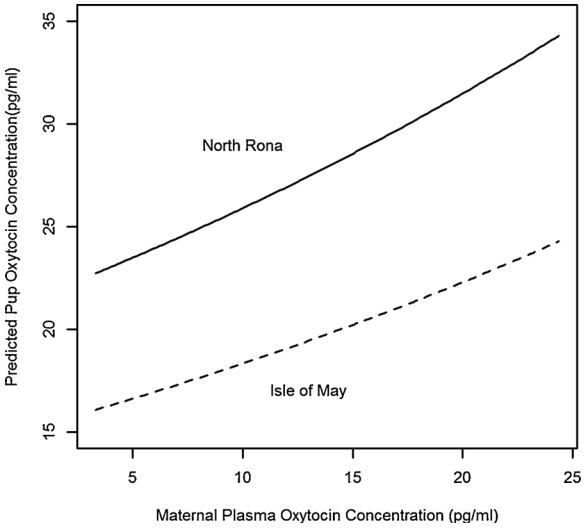

Fig. 2. Mother - pup plasma oxytocin relationships. Prediction plot showing the GAMM output of the relationship between mother and pup plasma oxytocin concentration (pg/ml) on North Rona (solid line) and the Isle of May (dashed line).

3.2. Maternal presence vs. Milk OT as drivers of high infant OT

To explore whether maternal presence may be driving elevated OT levels in pups, samples of plasma OT from as many pups as possible were collected after weaning, when mothers were absent during the natural 1–4 week post-wean fast that occurs in this species (Reilly, 1991). Pups that had weaned from their mothers had significantly lower plasma OT concentrations (10.9 ± 0.9 pg/ml) than when they were with their mothers in both early or late lactation (Fig. 3, ANOVA: $F_{2,126} = 37.18$, $p < 0.001$, Tukey honest significant difference test, $p = 0.5$ between early and late pup groups and $p < 0.001$ between weaned pups and all non-weaned pup groups).

To explore whether pups may be ingesting and absorbing OT from their mothers' milk, milk samples were collected from as many grey seal mothers as possible (n = 2) to estimate concentrations of OT that pups ingest from milk consumption. The two milk samples collected

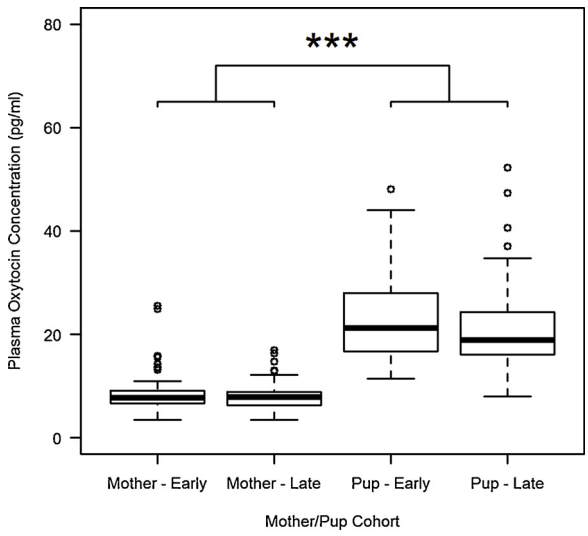

Fig. 1. OT concentrations in mothers and pups. Mean basal plasma oxytocin (pg/ml) in grey seal mothers and their pups during early and late lactation with median, upper and lower quartiles, 1.5x interquartile range and outliers shown. Significant differences at the $p < 0.001$ level between groups are denoted by asterisks.

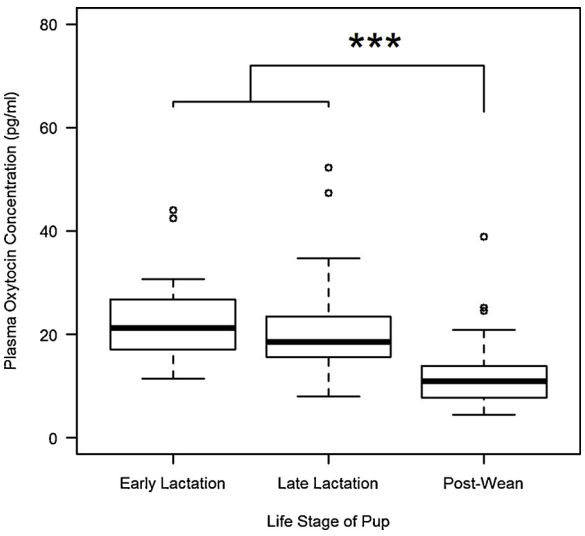

Fig. 3. Maternal presence as drivers of high infant OT. Mean basal plasma oxytocin (pg/ml) pups during early lactation, late lactation and post-weaning with median, upper and lower quartiles, 1.5x interquartile range and outliers shown. Significant differences at the $p < 0.001$ level between groups are denoted by asterisks.

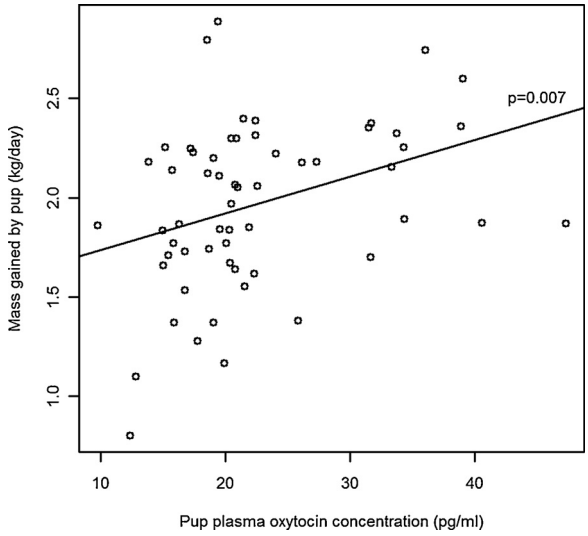

Fig. 4. OT concentrations and pup mass gain rate. The significant positive relationship between pup plasma oxytocin concentrations (pg/ml) and the mass a pup gains per day while still with its mother (kg/day) with the Pearson's correlation significance value.

contained 128.9 and 95.6 pg/ml OT, giving a mean of 112.2 ± 16.6 pg/ml (SE) in phocid milk.

3.3. OT concentrations, maternal mass loss and pup mass gain rate

Pup mass gain rate was linked to mean pup plasma OT concentrations across the lactation period (GAMM: R2 = 0.38, p = 0.016, Appendix B. Table B.2) with the two being significantly positively correlated (r = 0.35, p = 0.007, 95% CIs [0.1, 0.6], Fig. 4). A mother's rate of mass loss was independent of maternal OT concentrations in both early and late lactation (GAMM: R2 = 0.31, p = 0.17 and p = 0.11 respectively, Appendix B. Table B.3).

4. Discussion

4.1. High OT mothers produce high OT pups

The results for this study support the existence of positive OT feedback loops within mothers and pups in both of the seal colonies studied. Maternal and pup plasma OT concentrations were significantly higher on average than those detected in non-breeding female grey seals (4.3 ± 0.5 pg/ml, Robinson et al., 2015a), but there was great variation in individual values, especially within pups. Data on infant plasma OT levels are currently scarce, however, two studies measuring newborn OT plasma levels exist for humans and laboratory mice that mirror the OT patterns reported in this study. Human newborns had elevated plasma OT concentrations compared to adults in a study monitoring them for the first 4 days of life (Leake et al., 1981), while weaned human children have plasma OT concentrations comparable to those in adults (children 6–11 years: 1.2 pg/ml (Modahl et al., 1998), adults: < 2 pg/ml (Szeto et al., 2011). Laboratory mice pups approaching and at the point of weaning also have high plasma OT levels compared to other developmental stages (Higashida et al., 2010). Elevated OT levels are known to trigger proximity seeking behaviours in adult and infant grey seals (Robinson et al., 2015a; 2017). If stimuli from the presence of the mother/pup is causing the high OT concentrations recorded across the pair, the mother-infant positive feedback loop system proposed by Rilling and Young (2014) can be constructed with our data from a natural population (Fig. 5).

By documenting infant OT concentrations alongside their mother's levels, we provide the first evidence, to our knowledge, of double OT loops in mother-infant pairs, with one loop in each individual but dependent on each other's presence for their continuation (Fig. 5). Such loops would act to keep mothers and offspring together, synchronising them behaviourally and physiologically towards the common goal of infant survival. The structure and function of OT is widely conserved across the mammalian clade (Gimpl and Fahrenholz, 2001; Feldman et al., 2016; Jurek and Neumann, 2018). Thus far, grey seals have been shown to posses an OT system that is directly comparable to other domestic or captive animal species and humans, as their basal plasma concentrations, plasma clearance rates and maternal patterns of plasma OT expression match those detected in laboratory model species and humans (Robinson et al., 2014; 2015). Therefore, it is likely that the evidence for positive OT feedback loops across mother-infant pairs from grey seals would be present in other species.

The relevance of peripheral OT concentrations compared to central OT concentrations, and whether any meaningful correlations exist between the two is still debated (Valstad et al., 2017). However, peripheral and central release of OT due to stimuli from dependent infants has been documented in humans and rodents, including nursing, sounds and sight of the infant and interacting with the infant (Strathearn et al., 2009; Uvnäs-Moberg et al., 1998). Peripheral OT concentrations are also arguably more relevant to measure when investigating links between the hormone's concentrations in relation to mass changes in peripheral tissues, such as adipose deposits or skeletal muscle.

4.2. Maternal presence as a driver of high OT in pups

Our study found that pup plasma OT concentrations remain consistently high throughout the dependent period, only decreasing once they weaned and the mother was no longer present. A pup's developmental stage and the fasting state weaned pups enter as soon as the mother leaves could theoretically influence plasma OT levels. However, OT concentrations in individual grey seal pups show no variation across two weeks of fasting (Robinson et al., 2015b) and remain consistent when pups leave the breeding colony and start feeding at approximately one month of age, and throughout their first year of life (8.3 ± 0.6 pg/ml, Robinson et al., 2014). There is also no change in plasma OT levels across the various developmental stages either side of weaning, as levels in newborns are comparable to pups approaching weaning (see results Section 3.1), pups that have been fasting for 3 days are comparable to those who have fasted for several weeks (Robinson et al., 2015b) and fasting pups are comparable to all other developmental stages in the first year of life (Robinson et al., 2014). Pup OT decreases significantly and consistently in the first three days of the mother leaving, regardless of the age at time of weaning (Robinson, 2014). Pup plasma OT levels are subsequently stable for weeks despite undergoing sustained fasting and substantial developmental changes, and do not change as pups shift from fasting to feeding or undergo all the developmental changes that occur in their first year. It is more likely that some aspect of maternal presence is driving elevated OT in dependent pups, because once the mother leaves and this stimulus is removed, pup OT levels fall.

Ingestion of OT from breast milk has been proposed as a route of neonatal exposure to this hormone in humans (Uvnäs-Moberg et al., 1998; Carter, 2003) and mice (Higashida et al., 2010). Higashida et al. (2010) attribute ingested milk as the cause of high OT levels in mouse pups due to the sheer quantity of OT present in mouse milk. However, this interpretation must be viewed with caution, as the OT levels reported from that study indicate that unextracted substrates were used in the analysis which gives high, inaccurate results (Robinson et al., 2014; Leng and Sabatier, 2016). Higashida et al. (2010) also only detect the amount of OT in mouse milk, without putting this value into context with how much mice pups actually drink. Other studies state that physical barriers to absorption and uptake and chemical degradation in the digestive tract make ingested milk an unlikely source of significant amounts of OT in infants (Fjellestad-Paulsen et al., 1995). Even when

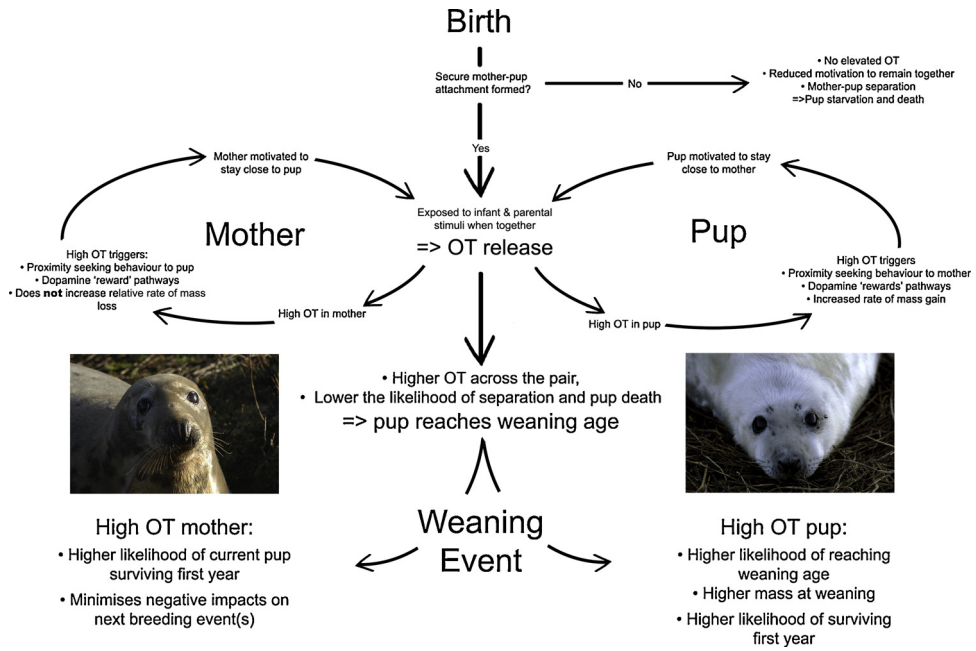

Fig. 5. Positive mother – infant OT loops and infant mass gain. Proposed double positive feedback loop involving oxytocin (OT) release, mother-pup bonding and behaviour and mass changes in grey seals.

medical trials have given high buccal doses of OT to humans, their ability to raise plasma OT concentrations is limited (Dawood et al., 1980; Landgraf, 1985). When put into context with the OT we detected in seal milk and the volumes of milk a seal pup ingests daily, it is apparent that the OT levels in seal milk are not high enough to impact on plasma concentrations (see Appendix C and Table C.4 for these calculations). The low number of milk samples that were obtained (n = 2) is a potential limitation of this study, as additional mothers may yield samples with higher OT concentrations. However, even if the questionably high OT levels detected in laboratory rat milk from Higashida et al. (2010) is used to calculate whether ingested OT could impact on pup plasma levels, these milk concentrations are still far too low to raise infant plasma levels significantly (see Appendix C and Table C.4 for these calculations). It is therefore plausible that aspects of the mother's presence other than her ability to provide milk are driving elevated OT in pups, potentially including the scent, sounds and sight of the mother.

Conspecific stimuli from individuals that are bonded to each other have already been shown to cause elevations in peripheral OT (Strathearn et al., 2009; Nagasawa et al., 2015). Other findings from this study also lend support to the theory that it is a mother's presence driving high pup OT levels. The inter-colony differences in mother-pup OT levels show that NR mother-pup pairs had significantly higher plasma OT than IoM pairs. Mothers on NR spend more time in close proximity to their pups than mothers on IoM (Redman et al., 2002) primarily due to topographical differences at the two colonies affecting access to water (Caudron et al., 2001; Redman et al., 2002). According to the positive loop theory, more time in close proximity equates to greater OT release and concentrations in bonded individuals, and the OT results from the two colonies agree with this (Fig. 2).

An endocrinological system that stimulates synchrony of both physiology and behaviour across individuals has the potential to act on other important bonds outside of maternal ones. There is evidence from social insects that complex social traits evolve from co-opting systems acting on maternal behaviour and physiology (Amdam et al., 2006), and it seems likely this has happened with the positive OT loop mechanism. There is already direct evidence that positive OT loops stimulate pro-social behaviour and elevate OT concentrations across socially bonded, but unrelated pairs even across species boundaries (Nagasawa et al., 2015). Therefore, this unique mechanism could enable the co-ordination of a number of individuals' physiology, across pairs or groups. By aligning group members' motivation to perform specific behaviours, OT may stimulate group synchrony even when faced with individual risks such as serious injury or death (Samuni et al., 2016). The existence of co-operative behaviour has generated much research into theoretical reasons for its development and perpetuation in individuals, populations and species; however, the underlying physiological mechanisms driving such behaviour remain relatively poorly understood (Soares et al., 2010). The OT loop system acting both within individuals and across group or bond members is a promising area for future work, uncovering how individuals can be motivated to act against their own interests in high risk or low reward contexts.

4.3. High OT pups gain mass faster

High OT concentrations were associated with greater pup growth rates without extra energetic cost to their mothers, as no differences in relative maternal mass loss rates were detected. Two results suggest that the difference in mass gain rates between high and low OT pups is not due to variation in how much milk pups ingest. First, behavioural data was collected from the NR mother-pup pairs in this study, and their plasma OT concentrations showed no relationship with variation in nursing bout frequency or duration (Robinson et al., 2015a). Second, if high OT pups were achieving their additional mass gain by ingesting more milk from their mothers, those mothers would show greater mass loss rates per day than low OT mothers, which was not observed. OT is known to modulate feeding in mammalian species (Gaetani et al., 2010; Atasoy et al., 2012) and has been shown to reduce food intake in several animal species (reviewed in Olszewski et al., 2010). This may explain why infants with elevated OT concentrations are not motivated to nurse more from their mothers. However, it does not explain how high OT infants are able to gain mass at a higher rate, without ingesting additional milk.

The variation in mass gain across high to low OT pups may be due to behavioural differences impacting individual metabolism and fat accumulation in pups. The elevated OT concentrations in pups are likely indicative of successful mother-pup attachment, and elevated OT would trigger pups to remain close to their mothers (Robinson et al., 2015a,

2017). This may reduce energetic expenditure in pups by preventing excursions away from their mother, which would elevate metabolic rate and initiate conflicts with adjacent seals. It is also possible that by encouraging pups to remain close to their mothers, high OT pups are more sheltered from strong winds (McCafferty et al., 2005), experiencing a microclimate that reduces their thermal output and lowers metabolic overheads. OT manipulations in laboratory rats indicate that the hormone triggers huddling behaviour (Alberts, 2007) and modulates the function of brown adipose tissue, directly impacting on thermoregulation in infants (Harshaw et al., 2018). Therefore, rather than actively stimulating mass gain, elevated OT concentrations in pups may reduce activities that divert resources away from growth prior to weaning.

With the growing body of evidence linking OT to the development of several tissue types, it is also possible that elevated OT in pups stimulates physiological pathways that cause increased mass development. Experiments giving OT to rat pups promoted weight gain in adults via increased deposition of adipose tissue (Uvnäs-Moberg et al., 1998) and when given to young pigs (*Sus scrofa domesticus*), OT reduced mass lost during weaning events (Rault et al., 2015). OT has also been linked to skeletal muscle development in mice (*Mus musculus*) (Elabd et al., 2014) and bone mass accumulation in mice and humans (Colaianni et al., 2015). Physiological pathways for increased OT concentrations influencing mass changes independent of food intake have been proposed (Rault et al., 2015; Colaianni et al., 2015), such as OT causing the stimulation of digestive activity and fat storage by linking increases in plasma cholecystokinin, insulin and adipose tissue in OT treated rats (Uvnäs-Moberg et al., 1998). More research is needed to identify which biological tissues are affected by OT, so that the developmental consequences for exposure to high or low OT levels due to variation in social or parental stimuli can be determined.

Grey seal mothers fast while nursing their pups and lose up to 40% of their body mass at parturition during this time (Pomeroy et al., 1999), using approximately 80% of their energetic reserves to produce milk and sustain themselves on the colony (Fedak and Anderson, 1982). The ability to wean at as large a mass as possible is the most important factor affecting grey seal pup survival in its first year of life (Hall et al., 2001). That OT facilitates mass gain or slows mass loss in dependent pups with no additional energetic cost to the mother is of great importance in a true capital breeding species which has rapid offspring mass gain and abrupt termination of maternal care. Any physiological factors enabling efficient mass gain in infants will be highly selected for as it would increases the probability of success for a mother within that breeding episode without additional investment costs.

Steady mass gain postpartum is crucial for successful infant development and survival in all animal species, including humans (Black et al., 2013; Shields et al., 2012). Any factors that increase infant mass gain while minimising the energetic costs to parents is highly advantageous in any species exhibiting parental care. All organisms must give their offspring the best developmental start in life while attempting to balance the negative costs to themselves; any factor reducing the conflict between these two contrasting demands on an organism will impact on their survival, their current and future reproductive success. A link between good maternal care, high OT and increased infant mass gain has been previously proposed in rodents based on manipulating OT levels experimentally (Uvnäs-Moberg et al., 1998). Additionally, a study investigating weight gain and massage therapy in preterm human babies theorised that elevated plasma OT in babies receiving massages indicated a role for the hormone in mediating infant weight gain (Field, 2001). To our knowledge, our study provides the first evidence of an OT-mass gain relationship in wild mother-infant pairs and highlights the importance of understanding the hormone's role in mediating mother-infant bonds, care giving behaviour and physical development in infants.

5. Conclusions

Our study provides the first evidence that positive OT loops acting across bonded individuals exist in mother-infant pairs in natural environments, and that they are linked to the promotion of infant development without additional energetic costs to mothers. Including energetic benefits in the proposed loop mechanism highlights how such systems physiologically give selective advantage to securely bonded mother-infant pairs (Fig. 5). OT facilitates and regulates parental and social bonds throughout the mammalian clade, with OT-like peptides in bird (Chokchaloemwong et al., 2013) and fish (O'Connell et al., 2012) species fulfilling similar roles in other vertebrate groups. OT loops and the associated fitness benefits linked to them may therefore be a widespread mechanism for connecting optimal parental or social environments with direct physiological advantages for individual development. Understanding the mechanisms by which OT and OT–like peptides affect interactions between the bonded individuals and infant mass gain has wide ranging implications for animal husbandry practises, medical interventions, advice to human parents, societal understanding of how health and relationships are linked and studying the energetic constraints of parental care.

Ethical approval

All animal procedures were performed under the UK Home Office project license #60/4009 and conformed to the UK Animals (Scientific Procedures) Act, 1986. All research received prior ethical approval from the University of St Andrews Animal Welfare and Ethics Committee and the School of Biology's Ethics Committee.

Data availability

The dataset supporting the conclusions of this article is included within the article and its Appendices (Appendix D).

Authors' contributions

KJR conceived the study; KJR, SDT and PPP collected samples in the field; KJR performed all sample and data analysis; NH provided essential laboratory equipment; KJR wrote the manuscript; all authors critically revised the manuscript and gave final approval of the version to be published.

Declaration of Competing Interest

None.

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data.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.104423.

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