Hormonal control of birth behavior in the Tasmanian devil

Sarcophilus harrisii

R.W. Rose a,⁎, L. Bell a, G. Shaw b

a School of Zoology, University of Tasmania Private bag 5, Hobart TAS, 7001, Australia
b Department of Zoology, The University of Melbourne, Victoria 3010, Australia

Received 23 December 2005; revised 11 May 2006; accepted 11 May 2006
Available online 11 July 2006

Abstract

In a number of marsupial species, females exhibit characteristic, stereotyped parturient behavior that facilitates the passage of the neonates to the pouch. In macropodids, this parturient behavior can be induced in non-pregnant females and males by treatment with either prostaglandin F2α (PGF2α) or oxytocin (OT). This study investigated the effects of PGF2α and OT on behavior of Tasmanian devils. Animals tended to sit or lie down quickly, with little vocalization, after treatment with PGF2α or OT, while after saline, the animals remained alert, seldom sat, and frequently vocalized. Hormone treatment caused increased respiration. Urogenital and pouch grooming, a characteristic element of parturient behavior in macropodids, was seen in only one devil after hormone treatment. However, no pouch or urogenital grooming was seen in videotape of a devil giving birth, so this may not be a feature of parturient behavior in this species. Overall behavior of males and females was very similar suggesting that the behavioral effects observed may be due to direct neural action of PGF2α or OT, rather than an indirect response to uterine or vaginal contractions caused by the hormones. This study is the first to demonstrate that OT results in PGF2α secretion as PGFM levels rose after OT injection.

© 2006 Elsevier Inc. All rights reserved.

Keywords: Marsupial birth behavior; Prostaglandin F2α; Oxytocin; Tasmanian devil

Introduction

Imminent birth in marsupials is heralded by species-specific stereotyped parturient behaviors that include positioning of the body and tail and grooming of the pouch (Sharman and Pilton, 1964; Sharman and Calaby, 1964; Renfree et al., 1989). This behavior is only seen in the minutes around birth and is caused by hormonal changes associated with parturition. In tammar wallabies, where the endocrine changes at birth are well understood (reviewed in Renfree et al., 1997a,b; Shaw and Renfree, 2001), both prostaglandin F2α (PGF2α) and oxytocin (OT) are crucial for delivery of the fetus. Plasma concentrations of both hormones rise to a sharp peak within minutes before delivery. PGF2α also controls aspects of the birth behavior that are characteristic of many marsupial species. This behavior starts within a few minutes after injection of PGF2α and can be induced in both females and males, suggesting that PGF2α acts via a direct action on the central nervous system (Hinds et al., 1990; Shaw, 1990; Gemmell, 2002; Gemmell et al., 1991; Rose and MacFadyen, 1997; Smith et al., 2001; Rose and Fadem, 2000).

We have also shown that OT causes this behavior but with an increased latency after injection and, as this response fails to occur after injection of a PG inhibitor, we hypothesized that OT stimulated the secretion of PGF2α that then induced parturient behavior (Rose and MacFadyen, 1997).

In this study, we have investigated the potential role of PGF2α and OT in the control of birth behavior in the Tasmanian devil (Sarcophilus harrisii). The Tasmanian devil is a medium-sized carnivorous marsupial only found on the island of Tasmania, off southeast Australia. It has a short breeding season early in the year (March), and its gestation length is approximately 19 days (Rose, 1989; Guiler, 1970a,b; Hughes, 1982). The birth behavior of the Tasmanian devil has been documented only once, in a television documentary entitled ‘The Devil’s Playground’ (NHNZ, 1999). In this documentary, the birth posture shown involves the female devil standing on all four legs, with her back arched and her head resting on the ground (Fig. 1).
This posture creates a downward path for the young from the urogenital opening to the backward opening pouch. Birth behavior in the family Dasyuridae has been poorly studied compared to that of other marsupial families.

Birth behavior varies between marsupial families, but within a family the behaviors are often similar. Gemmell et al. (2002) classified birth behavior into three types based on the structure of the female’s pouch. Type I includes those marsupials in which the pouch is forward facing, such as the macropodids and possums. The birth position in these species results in the young having to climb up to the pouch from the urogenital opening (Fig. 1). Type II includes those marsupials with a backwards-opening pouch, such as the bandicoot. The birth position allows the young to travel downwards to the pouch from the urogenital opening. Type III, those with a pouch that develops as the young grow in size, includes the dasyurids. One species in this group in which birth has been observed is the northern quoll, Dasyurus hallucatus. The female was seen to stand on all fours, with the hind legs positioned higher than the forelegs. However, the movement of the young from the urogenital opening to the pouch was not observed (Gemmell et al., 2002; Nelson and Gemmell, 2003). The birth position in this species appears to be similar to that of the Tasmanian devil.

In this study, we assessed the behavioral responses of non-pregnant female and male Tasmanian devils to injection of PGF2α and OT. Since the behavioral response to OT injection may be mediated by prostaglandin (PG) production, a second series of devils were treated with OT and blood samples taken to assay concentrations of PGFM, the main PG2α metabolite (Shaw, 1983, 1990).

Methods

Animal husbandry

Animals were trapped over several nights during the mating season (February/March). The animals used in this study were all kept in accordance with the National Health and Medical Research Council of Australia Code of Practice for the Care and Use of Animals for Scientific Purposes. Approval was received from the University of Tasmania’s Ethics Committee for the study, and permission to trap the Tasmanian devils was obtained from the Department of Parks and Wildlife. All Tasmanian devils that were retained after trapping were kept in four large outdoor enclosures at the Animal House, University of Tasmania. The enclosures were each approximately 3 m wide, 6 m long and 1.8 m high, with grass/soil bottoms. One half of each enclosure was covered on all sides with galvanized iron for protection from the weather. Straw was provided for the devils as nesting material. At least one sleeping box per animal was provided. The female devils often shared a sleeping box, while the males only occasionally shared a sleeping box.

Up to three devils were kept in each enclosure. Males and females were kept in separate enclosures to prevent pregnancies, except for one female that was kept with a male specifically to facilitate mating as part of another unrelated study. Animals were held for at least 6 weeks after trapping before experimental use. Individual devils were identified by their unique white pelage markings.

Water was provided in plastic or metal bowls, and these were cleaned and refilled daily. The animals were fed approximately every 2 days on meat and bone off-cuts from a local butcher, wallaby meat, rabbit carcasses and rats. They received between 4 and 6 kg of food each per week, depending on the mass of the devil.

One of the females and one of the males used for behavioral studies were captive-bred animals that were made available for use by the Tasmanian Devil Park at Taranna, on the Tasman Peninsula. These animals were used for behavioral study only and were treated and observed in their own enclosures, in order to determine if the behavioral responses might be more intense in animals that were accustomed to captivity and handling, and not disturbed by a change of enclosure.

Effect of PGF2α and OT on behavior

Six females and four males were used for behavioral studies. Three of the females were juveniles, less than 2 years old, and weighed between 4 kg and 5 kg. The remaining females were adult, one weighing 7.5 kg, and the other 5.5 kg. The three males were all adult and weighed between 6 kg and 10 kg.

One of the four housing enclosures was modified for use as the observation area. One end of this enclosure was cleared of sleeping boxes and other obstructions to viewing. During the observation period, the entrances to the sleeping boxes were blocked to keep the devils in open view. The devils used were transferred to this enclosure at least 1 day before the beginning of their part in the experiment.

Devils were treated with PGF2α (Lutalyse, 5 mg/ml, Pharmacia, Upjohn) at a dose rate of 0.1 mg/kg body weight i.m. of PGF2α as in Smith et al. (2001). OT (Ilium Synthecinon, 10 i.u./ml, Troy Laboratories) was given at a rate of 1 i.u./kg i.m. As a control, an injection of 0.1 ml saline (sodium chloride injection, BP 0.9% parallels 1000 ml, Baxter Healthcare) was administered i.m. To administer a treatment, a devil was transferred from its sleeping box to a hessian bag by picking it up by the base of the tail. The animal was then restrained by placing a hand behind the head, pinning the head to the ground. Further pressure was placed on the body if required.
The sampling times where blood was obtained are clear from Fig. 4. It was not possible to obtain a sample from all animals at each planned time point. Generally remained relaxed throughout blood sampling. Due to technical problems, open cuts. The devils showed no signs of discomfort during the procedure and devil’s ear, rubbing of the cut with a clean swab or flicking the ear were used to re-open cuts. The devils showed no signs of discomfort during the procedure and were collected in heparinized capillary tubes to prevent the blood from clotting. Between 600 and 800 μl of blood was collected per sample on most occasions. The plasma was then extracted and stored at −20°C until analysis.

Blood samples were taken before about 10 min before an injection of either OT (1 i.u./kg body wt) or saline (0.1 ml), immediately after the injection, and then at 10, 20, 40 and 60 min after the injection. To minimize the number of cuts made to each devil’s ear, rubbing of the cut with a clean swab or flicking the ear were used to re-open cuts. The devils showed no signs of discomfort during the procedure and generally remained relaxed throughout blood sampling. Due to technical problems, it was not possible to obtain a sample from all animals at each planned time point. The sampling times where blood was obtained are clear from Fig. 4.

**Effect of OT injection on plasma PGFM concentrations**

Three adult male, one adult female and 3 juvenile female devils were used in this experiment which commenced 2 weeks after the end of the previous study. These were the same devils previously used in the behavioral studies. Devils were transferred to hessian bags as described above for the duration of blood sampling. An ear was exposed through the bag opening, and blood samples were obtained by using a scalpel blade to nick the peripheral ear vein. The blood droplets were collected in heparinized capillary tubes to prevent the blood from clotting. Between 600 and 800 μl of blood was collected per sample on most occasions. The blood from each sample was then transferred from the capillary tubes to a 1.5-ml Eppendorf® tube by flicking the capillary tubes. Blood samples were kept chilled until centrifuged. The plasma was then extracted and stored at −20°C until analysis.

Blood samples were taken before about 10 min before an injection of either OT (1 i.u./kg body wt) or saline (0.1 ml), immediately after the injection, and then at 10, 20, 40 and 60 min after the injection. To minimize the number of cuts made to each devil’s ear, rubbing of the cut with a clean swab or flicking the ear were used to re-open cuts. The devils showed no signs of discomfort during the procedure and generally remained relaxed throughout blood sampling. Due to technical problems, it was not possible to obtain a sample from all animals at each planned time point. The sampling times where blood was obtained are clear from Fig. 4.

**PGFM assay**

PGFM was assayed in 50 μl plasma aliquots using the method of Lewis et al. (1986). All samples were processed in a single assay. Assay sensitivity was 100 pg/ml and the within assay coefficient of variation was 16.7%.

**Statistical analysis**

The times until the first expression of particular behaviors associated with birth were analyzed using repeated measures analysis of variances using the log-transformed data. Video footage was to be used to compare other aspects of birth behavior. A repeated measures analysis of variance was first conducted on log plasma PGFM concentration. The combined control and experimental values were tested to determine if there was any difference in the responses of the two sexes. As there was no significant difference between the sexes, a repeated measures analysis was then undertaken on the combined results from the two sexes in order to determine whether the treatment affected the levels of PGFM and also whether PGFM levels changed with time after the injection.

**Results**

**Behavioral responses to PGF and OT**

After treatment with saline, both male and female devils behaved in a manner typical for this species. The animals tended to pace about and occasionally jumped, but as they were unable to enter their nest boxes, they remained in positions in which they could keep the observer in clear view, and where a defensive move or display could be executed effectively. The earliest that an animal was observed sitting or lying after control injection was at 15.5 min, and the timing for males and females was similar (Fig. 2). Once the animals sat or lay they tended to remain in that posture for prolonged periods. In contrast, after either PFG2α or OT treatment the animals appeared calmer and tended to adopt less defensive postures. Throughout the observation period, they would adopt a sitting or lying posture for a few minutes before restlessly moving standing and moving to a new posture. Most animals treated with either PFG2α or OT sat or lay within minutes of injection (Fig. 2) mainly with their hind limbs extending forward (Figs. 1b and c). The onset of sitting or lying was significantly quicker with females than males after both PFG2α and OT (Fig. 2). Although the animals sat or lay relatively soon after PFG2α or OT injection, they always maintained a position where they had clear view of the observer. The 2 adult females

![Fig. 2. The mean time (± SEM) taken for male (gray bars) and female (open bars) devils to sit or lie after injection of saline, PFG2α or OT. Analysis of variance of the log-transformed data indicated a significant effect of treatment and a significant treatment by sex interaction due to the more rapid onset of sitting or lying in females after treatment. Asterisks and braces indicate significant differences (specified contrasts within the analysis of variance). *P < 0.01.](image)

![Fig. 3. Time to first vocalization (mean ± SEM) after injection of saline, PFG2α or OT. Males (shaded bars) were similar to females (open bars) after saline (control) or PFG2α treatment, but after OT treatment males, unlike females, showed no inhibition of vocalization. Asterisks and braces indicate significant differences (specified contrasts within the analysis of variance). *P < 0.05.](image)
appeared notably restless after PGF2α. They rarely remained in the same position for more than a few minutes, repeatedly lying down apparently comfortable but then rose, moved slightly and lay down again throughout the observation period. Restlessness to a lesser extent was also noted in the juvenile females after PGF2α injection, but such restlessness was not noted in males or in females with saline or OT treatment. Hormone treatment also altered vocalization behavior. Most control treated devils made vocalizations within 5 min of injection, and there was no difference in this timing between males and females (P > 0.05, Fig. 3). Many of the PGF or OT treated animals either had substantial delays in vocalization time, or failed to vocalize during the observation period (Fig. 3). This delay was highly significant overall (P < 0.002), but there was a significant sex x treatment interaction (P < 0.003). This interaction is due to a significant sex difference after OT treatment. Males vocalized soon after treatment, whereas treated females had delayed onset of vocalization.

Two captive-bred devils from a Tasmanian Devil Park were included in order to establish whether or not the stress of a new environment was the reason the wild-caught devils had not exhibited full birth behavior. The captive-bred devils were used to regular handling and were observed in their own enclosures, so they were not exposed to unfamiliar situations; these devils were presumed to be under minimal stress. However, the results from these two devils were very similar to those of the wild-caught devils, except that the captive-raised female after treatment with PGF2α and OT groomed its urogenital opening, a behavior not seen after saline injection, or after any treatment in any of the other devils. No grooming was observed throughout the experimental or control trials, except in the captive-bred female from the Tasmanian Devil Park, that repeatedly cleaned her face with her forepaws, and on two occasions briefly cleaned her urogenital opening.

Aside from behavioral changes, there were also physiological signs that the PGF2α and OT were affecting the devils. While the devils in this study did not pant in an obvious manner following saline injections, an increased respiratory rate was observed after either PGF2α or OT injection in all devils and on 4 occasions fluid was seen dripping from the nose and/or mouth.

Plasma PGFM concentrations after OT injection

Plasma PGFM concentrations were highly variable. No significant difference was seen between males and females so sex was dropped as a factor in the analysis of variance. OT injection caused a significant increase in plasma PGFM between 10 and 40 min after injection (P < 0.05) (Figs. 4, 5), but there was no significant difference between males and females (P > 0.1) (repeated measures analysis of variance). One animal (J4) showed a sudden peak 60 min after saline injection. Overall, there was a very high between animal variation in assayed PGFM levels.

Discussion

Subsequent to these experiments, the Tasmanian devil has been diagnosed as suffering from a debilitating and fatal disease known as the “Devil Facial Tumour Disease” (Jones et al., 2004, 2005; Bradshaw and Brook, 2005), although this study was conducted on animals before the disease became established, it is considered vital that research on this species be disseminated.

Treating both male and female Tasmanian devils with either PGF2α or OT induces behavioral changes that mimic some of the behavioral changes seen at birth including calmness, sitting or lying with the hind legs extended forwards, decreased vocalization, and increased respiration. These changes were not as pronounced as seen at parturition, and licking of the urogenital area was only seen...
with one captive bred female. Nevertheless, this partial birth behavior was strikingly different to the behavior of control, saline injected females.

The lack of full birth behavior in response to PGF2α in this study contrasts with our results in a pilot study (Smith et al., 2001). In that study, an individual male and female responded with birth behavior to PGF2α injections, but neither responded to OT. It is possible that the degree of behavioral response may vary between individuals, stress may play a part, but it is most likely that the significant difference was a result of our larger sample size. The parturient behavioral response of tammar wallabies that are accustomed to frequent handling is faster and more complete than the response of animals that are unused to handling (unpublished observations).

In our experiments, the hormone treatments induced changed behavior in both females and males. This is consistent with previous observations in other marsupial species and supports the idea that this is a direct neural response, rather than a response to uterine or vaginal contractions. Since adult males behave similarly to adult females in response to PGF2α in this and other marsupial species, it seems clear that these behavioral responses are not pre-programmed in a sex-specific way by gonadal hormone exposure during development, as is also the case with male sexual behavior in tammar wallabies (Rudd et al., 1996). The similarity of the responses to both PGF2α and OT suggests that they may potentially act through the same ultimate pathways. Male devils tend to be more vocal than females, but both responded similarly to the injection of PGF2α with delayed vocalization. The failure of OT injection to delay onset of vocalization in males but not in females may reflect a dose-sensitivity effect, with males less sensitive than females to OT or the PGF2α it induces. Alternatively, this may reflect a gender-specific difference as males give characteristic calls during courtship (Croft, 1982).

The significant decrease in the time taken for the devils to sit or lie for the first time in both the experimental treatments compared to the control is best interpreted in conjunction with the observations as a whole. The devil’s apparent lack of control over their behavior in the experimental treatments may be in part due to the psychoactive effects of these hormones in marsupials. Previous studies have shown that marsupials become oblivious to their surroundings, and even to handling by observers (Hinds et al., 1990; Shaw, 1990; Rose and MacFadyen, 1997; Smith et al., 2001). Lyne (1974) commented on how marsupial bandicoots while giving birth were oblivious to the presence of observers.

The decrease in vocalization by both sexes in response to the hormones compared to the control accompanied a decrease in respiratory rate and restlessness are among the known side effects of PGF2α when used to induce labour in horses (Klem et al., 1982), and these were seen in devils of both sexes during the PGF2α trials, and occasionally during the OT trials. Hinds et al. (1990) and Shaw (1990) all noted that in tammar wallabies after PGF2α administration, the animals panted during the time when they were most intensely exhibiting birth behavior. Although the devils did not pant in an obvious way, panting in devils is not always easily observed (Hulbert and Rose, 1972).

The restlessness noted after injection of PGF2α into females may be due to some discomfort caused by the action of the PGF2α on the uterine smooth muscle, as this reaction was not seen in any of the male subjects.

Although Tasmanian devils have been kept and bred in captivity successfully by a number of zoological institutions, observations of birth have been reported only once (NHNZ, 1999). Devils most probably give birth in a secluded, safe den, as seen in NHNZ (1999) and the open observation area used in this experiment may not have been adequate for them to fully express birth behavior in response to the hormone injections. It is also probable that devils give birth during the night, and as this experiment was conducted during the day, repeating the experiment either during the night or with the animals under reversed daylight conditions may allow more complete induction of parturient behavior.

The behaviors that we looked for during the observations were based on those exhibited by other marsupial species during similar experiments. However, S. harrisi is from an entirely different family to any other species studied so far, and as a result, it may not have been appropriate to expect these behaviors to be part of Tasmanian devil birth behavior. In other marsupial species studied, intense and frequent grooming of the pouch and urogenital opening is a common sign of birth behavior; however, in NHNZ (1999), the female did not groom any area of her body.

It is clear that birth behavior in response to PGF2α is most easily observed in captive animals that have been regularly handled (Rose and MacFadyen, 1997). The devils used in this series of experiments were not. However, the devils used in our pilot study (reported in Smith et al., 2001) were relatively tame and had been handled regularly as well as being acclimated to an injection regime (Kabat et al., 2003). Acute stress halts birth behavior and birth in mice and reduces OT levels to that of virgin mice (Douglas et al., 2002).

Both OT and PG are required for successful birth in marsupials (Renfree et al., 1994, 1996; Shaw and Renfree 2001; Gemmell, 2002). OT is not essential for parturition or reproductive behavior in mice (Nishimori et al., 1996). OT can induce uterine PGF2α release in eutherian species (e.g., Flint et al., 1994; Carnahan et al., 2002) but previously not demonstrated in a marsupial. As OT takes longer to induce parturient behavior in marsupials than PGF2α, and OT has no effect if preceded by a PGF2α synthesis inhibitor (Rose and MacFadyen, 1997; Smith et al., 2001), we tested the idea that OT was acting through increased release of PGF2α. In this study, we showed that administration of OT caused an increase in the PGF2α metabolite, PGFM, in peripheral blood. This increase was seen in both males and females so cannot be ascribed to release of PGF2α from the uterus. These data are consistent with previous studies suggesting that OT induces parturient behavior by causing a release of PGF2α. The one animal that showed a higher PGF2α value 60 m after saline injection may have responded to something other than the injection.

PGs and OT are also important for birth-associated behavior in some eutherian species. For example pigs display a characteristic
nest-building behavior in the day preceding parturition. PGF2α can induce this behavior, and treating females that have started nest-building with PG synthesis inhibitors suppresses the behavior (Gilbert, 2001; Gilbert et al., 2002). OT is well recognized as a major regulator of maternal behavior at the time of birth (e.g., Keverne and Kendrick, 1992; McCarthy et al., 1992; Pederson et al., 1982, 1992). PGs and OT may well act through similar pathways in eutherians and marsupials to regulate behavior in the peripartum period. Marsupials provide a valuable model for investigation of the role of PGs and OT in acute regulation of behavior at birth because of the lack of sex-specific developmental programming of these behaviors and the very stereotyped nature of these behaviors in some species.

Acknowledgments

We thank Associate Professor G. Jenkin, Monash University, Melbourne, for the supply of the PGFM assay tracer, and Professor A.P.F. Flint, University of Nottingham, for the PGFM antiserum used. We also thank Natural History New Zealand for the permission to include Fig. 1a and Mr. John Hamilton (Tasmanian Devil Park) for access to several Devils.

References


