Experimental peripheral administration of oxytocin elevates a suite of cooperative behaviours in a wild social mammal

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The evolution and expression of different forms of cooperative behaviour (e.g. feeding, guarding, sentinel duties, etc.) are usually studied independently, with few studies considering them as a single syndrome. However, studies investigating individuals’ investment across a suite of different behaviours reveal that they are correlated, suggesting a single mechanism determining the evolution and expression of cooperative behaviours. A hormonal mechanism could achieve this, and one possibility is oxytocin (OT), which affects several prosocial or alloparental behaviours independently. We show, using a double-blind experiment, that peripheral administration of OT to social, free-living meerkats Suricata suricatta elevates a suite of cooperative behaviours. Treated individuals increase their contributions to communal, cooperative activities (digging, guarding, pup-feeding and associating with pups) and decrease initiation of aggressive interactions, compared with a saline control. This suggests that different forms of cooperative behaviour form a single syndrome with a common causal basis. If our peripherally administered OT acts in the same way as the naturally released hormone, then a general tendency to prosociality may be modulated by this hormonal system. Therefore, it may be difficult for an individual to decouple expression of cooperative behaviours that provide the practitioner with benefits from those that provide the recipient with benefits. It may also explain why social species typically exhibit a suite of cooperative behaviours, without having to invoke independent evolution of each.

**Keywords:** cooperation; oxytocin; meerkats

1. INTRODUCTION

Non-breeding individuals of cooperatively breeding species contribute to a range of alloparental and prosocial behaviours that enhance the fitness of other group members, including the young of breeding relatives [1,2]. Most studies of such cooperative behaviours (feeding, guarding, sentinel duties, etc.) have considered individuals’ contributions to each to be determined on the basis of specific costs and benefits, and therefore independent of their investment in alternative cooperative behaviours (e.g. [3]). However, the few studies that have investigated individuals’ investment in a suite of different activities suggest that they are correlated [4,5]. This suggests that there may be a single mechanism determining investment across cooperative or prosocial behaviours. A hormonal mechanism could achieve this, and one possibility is oxytocin (OT), previously shown to affect several independent behaviours.

Oxytocin is a mammalian peptide hormone that acts on the central nervous system and is generally associated with uterine muscle contraction at birth and milk let-down in females. OT is also implicated in a range of behaviours not directly related to birth and early life. A range of prosocial behaviours across mammalian taxa are modulated by variation in production or reception of OT (e.g. social contact [6]; pair bonding [7]; parental care [8–11]; trust [12]; generosity [13]; social memory [14]). Previous work has concentrated on maternal or pair bonding between sexual partners or small family groups of captive animals, which naturally exhibit uniparental or biparental care. However, there is some evidence that variation in levels of OT or receptivity affects more general prosocial behaviour between individuals that are not sexual partners or parents and offspring, exemplified by alloparental care [15–17]. Such prosocial behaviour may be promoted by the structurally similar hormone vasopressin [18], or by an interaction between the two hormone systems [19]. In this study, we concentrate on the better-studied OT system.

Meerkats (Suricata suricatta) are a model species in which to look for natural alloparental and prosocial behaviours. They are cooperative breeders that live in groups of up to 50 individuals in which a dominant pair of adults obtain the majority of breeding [20]. Other adults in the group provide alloparental care by babysitting [21], feeding pups [22] and teaching pups correct foraging decisions [23]. Adults also contribute to other communal behaviours such as guarding [24], mobbing [25] and excavating burrows used for sleeping or escape [26]. Such prosocial behaviours have hormonal correlates [27,28].

We used a population of free-living meerkats to test whether experimental intramuscular administration of OT affects individuals’ behaviour. Specifically, we tested whether administration of OT had a general effect across a suite of prosocial behaviours, predicting that we would observe a coordinated increase in all forms of cooperative and prosocial behaviours when OT levels were increased.

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2. MATERIAL AND METHODS

(a) Study system
Thirty-six meerkats from four groups were studied in the Kuruman River Reserve, South Africa (26° 58′ S, 21° 49′ E) between April 2007 and February 2008. These comprised 28 males and 8 females. In the first two groups, only males were studied because of concerns over the effect of OT on female reproductive behaviour (see below). In the second two groups, all adults were studied. All experiments were conducted when groups contained pups aged between 40 and 60 days old, which corresponded to the peak period of pup provisioning [22]. Experiments were carried out after the meerkats had left their sleeping burrow and had been foraging as a group for at least 15 min. If the group engaged in an intergroup interaction, the experiment was halted. All animals were habituated to allow close observation (less than 1 m) and marked for individual identification with hair dye.

(b) Experimental protocol
Double-blind tests were made, and individuals acted as controls for themselves and as controls for another group member. The person injecting the meerkats did not know which treatment was being administered, and the observers did not know which of a pair of individuals had received what treatment. Individuals were paired—ideally with a littermate, but if this was not possible then with an individual differing in age by less than six months. One individual in each pair was randomly assigned to either the experimental or a control treatment, with its partner simultaneously receiving the alternative treatment. The treatments were reversed 3–5 days later, allowing sufficient time for any effect of the hormone to disappear. This ensured that each individual acted as a control for itself, such that its behaviour was recorded when subject to experimental and control treatments. It also allowed one individual to act as a control for the other so that changes in one individual’s behaviour could be compared across the two days of the experiment, during which time environmental and ecological conditions were unlikely to be constant.

Meerkats received an intramuscular injection. We used a 1 ml insulin syringe with a 29 g needle to inject into the rump of the meerkat while it was foraging and digging. Meerkats immediately sprang away from the injection, but there was no long-term effect of the physical penetration, such that all meerkats immediately resumed foraging in the same hole from where they had been disturbed and did not become wary towards the observers. They responded in a similar manner to bites by ants that they naturally encountered while foraging (J. R. Madden 2007, personal observation). Experimental animals received doses of OT (Pentocin Virbac; each 1 ml contained OT synthetic 10 IU and chlorobutanol 0.5% v/v). Dosage was calculated by extrapolating published recommended intramuscular dosages for a range of domestic animals to fit the body mass of meerkats [29]. This resulted in a dose of 0.01 ml 100 g⁻¹. Individual body masses were collected over the week prior to the experiment and rounded up to the nearest 100 g, allowing the appropriate dosage to be calculated. Control animals received an injection of the same quantity of saline solution.

After the treatment had been administered, individuals were followed by an observer at a distance of 2–3 m for 30 min. The time period was selected as this is the duration for which peripherally administered OT typically acts to induce labour. A scan was made every 30 s recording the activity engaged in and the presence of adults and pups less than 1 m away. This gave measures of common behaviours and affiliations as instances out of a possible 60. During each minute, we recorded: all aggressive events, including who initiated such events; all food items found, their size [22] and their fate, whether eaten or fed to a pup. This gave measures of less common behaviours as counts. We also recorded the total duration of all digging behaviour during the 30 min. An individual’s generosity in feeding pups was calculated by dividing the size-corrected number of food items that they fed to pups by the total size-corrected number of food items found.

(c) Statistical analyses
We tested the efficacy of the treatment by comparing the proportion of each activity that an individual performed relative to its partner, when the individual was treated with OT and when treated with saline. For example, individual 1 was initially treated with OT and was recorded as foraging for 40 of the 60 scan points, whereas its partner, individual 2, was initially treated with saline and also recorded as foraging for 40 of the 60 scan points. When the treatments were reversed 4 days later, individual 1 received saline and was recorded as foraging for 30 of the 60 scan points, and individual 2 received OT and was recorded as foraging for 10 of the 60 scan points. The gross differences in foraging levels (80 instances on day 1 versus 40 instances on day 2) are likely to be due to ecological factors, perhaps prey abundance or richness. On day 1, both individuals engage in 50 per cent (40/80) of the total foraging instances (TFI). On day 2, individual 1 engaged in 75 per cent (30/40) of TFI, and individual 2 engaged in 25 per cent (10/40) of TFI. Therefore, individual 1 engaged in 50 per cent TFI when treated with OT compared with 75 per cent TFI when treated with saline. Individual 2 engaged in 25 per cent TFI when treated with OT compared with 50 per cent TFI when treated with saline. Therefore, although individual 1 generally spends more time foraging than individual 2 (perhaps because of size, foraging inefficiency, prey specialization etc.), both individuals forage proportionately less when treated with OT than when treated with saline.

Such measures are effectively indices of behaviour and can tell us little about the effect size of the treatment. Therefore, we used non-parametric paired statistics to ensure a conservative comparison. For common behaviours that occurred more than 10 times/session, such as foraging and guarding, and association data, we used Wilcoxon signed-ranks tests. For uncommon behaviours that occurred less than seven times/session, such as pup feeding, digging and aggression, we used sign tests with a binomial distribution, meaning that we can only comment that such behaviours are elevated or depressed by the administration of OT. Owing to the small sample sizes and use of non-parametric statistics, we did not analyse the sexes separately. All analyses were conducted using SPSS and two-tailed tests were used throughout.

(d) Ethical note
Preliminary trials of the method and effects of OT and saline were conducted on a small number of individuals from groups not used in this experiment. We initially treated males, because of concerns over the effects of OT on female reproductive cycles. We then conducted trial treatments on three females. When our preliminary trials
revealed no effects on females, we expanded our experiment to include females. A vet was present for all of the preliminary trials and for the first experimental group. We continued to visit the meerkats following the treatments as part of other ongoing studies on the population and noted no long-term abnormalities in their behaviours or response to human observers. The injections did not cause any infections or injury. We analysed the results of the experiment after each group, and when significant effects of the treatment were revealed we ceased the experiment so as to minimize the number of meerkats treated.

3. RESULTS

Individuals treated with OT performed prosocial behaviours at higher levels than when treated with saline. OT-dosed individuals spent more time on guard (Wilcoxon signed-ranks test: $n = 36$, $Z = 1.95$, $p = 0.05$; figure 1a). OT-dosed individuals were more generous in the proportion of food that they fed to pups (sign test: $n = 36$, $p = 0.027$; figure 1b). OT-dosed individuals spent more time close to pups (Wilcoxon signed-ranks test: $n = 36$, $Z = 2.79$, $p = 0.005$; figure 1c), but not more time next to adults (Wilcoxon signed-ranks test: $n = 36$, $Z = 0.90$, $p = 0.37$). OT-dosed individuals initiated fewer aggressive events towards group members (sign test: $n = 36$, $p = 0.023$; figure 1d). OT-dosed individuals spent longer in digging at burrows and bolt holes (sign test: $n = 36$, $p = 0.004$; figure 1e).

This increase in prosocial behaviour when treated with OT was offset by a tendency to decrease time spent foraging compared with the individuals given the saline treatment (Wilcoxon signed-ranks test: $n = 36$, $Z = 1.82$, $p = 0.068$; figure 2a). This resulted in less food being eaten when an individual was treated with OT (Wilcoxon signed-ranks test: $n = 36$, $Z = 2.67$, $p = 0.008$; figure 2b).

4. DISCUSSION

Experimental administration of OT caused meerkats to engage in higher levels of prosocial behaviours that were directed towards both pups (feeding generosity and close association) and adult group members (guarding, digging and decreased aggression). Thus, in meerkats,
administration of peripheral OT modulates a general suite of prosocial behaviours. This extends previous work on humans and captive rodents showing that OT promoted specific prosocial behaviours. Unlike captive rodents, free-living meerkats habitually exhibit natural alloparental and prosocial behaviours, being cooperative breeders that live in groups.

It is surprising that peripheral administration of OT had such an effect. Most previous studies demonstrate that OT acts on central nervous system receptors in brain regions which are isolated from the peripheral circulatory system by the blood–brain barrier. Because we did not sacrifice individuals, we cannot describe which receptor sites our administered OT interacted with, nor can we quantify levels, or changes in levels, of OT in peripheral or central circulation. Nevertheless, our observation of behavioural change demands explanation. We can suggest two possible mechanisms that explain why our intramuscular injections had effects similar to those known to be mediated by central receptor systems. First, despite the relative impermeability of the blood–brain barrier, peripherally administered OT can be detected in the brain, with small amounts (approx. 1%) of that administered crossing the barrier (e.g. [30]), and this can have long-term effects on the expression of vasopressin receptors in brain regions [19]. Our dosage was calculated based on recommendations for intramuscular injections required to induce labour. Such levels may be sufficiently high to permit a small percentage to cross the blood–brain barrier, enough to directly stimulate OT receptors in the brain. Second, our injections may act directly on receptors in the periphery, which in turn promote a neural feedback stimulating central effects. Such receptors could also include those responsive to arginine vasopressin, a very similar neuropeptide also implicated in prosocial behaviour (e.g. [18]). In such circumstances, it is possible that the prosocial behaviour was not directly mediated by OT, but instead by other hormones or neurotransmitters released centrally following our peripheral administration of OT. A likely alternative candidate could be vasopressin. Ideally, we would have administered an OT antagonist directly into the central system and observed a predicted fall in the same suite of prosocial behaviours. Logistical constraints prevented this. Regardless of the exact mechanism of operation, the observed change in individual prosocial behaviour requires a functional explanation.

Such general prosociality in meerkats brings direct benefits to individual practitioners: increased time on guard reducing a practitioner’s susceptibility to predators [24]; improved burrows offering the practitioner shelter from predators [26]; and thermal protection to the practitioner from extreme temperatures [3]. Prosocial behaviours also bring indirect (kin-selected) benefits to the practitioners by increasing the direct fitness of other (usually closely related [20]), group members. Feeding and caring for pups improve their likelihood of survival [31] and attaining dominance [32]. Other adult group members benefit in similar ways to the prosocial individual through access to common goods in the form of a system of well-maintained and prolific shelter burrows [26], and from improved predator detection and alarms given by guarding individuals [24]. Owing to the use of non-parametric statistics with small and unbalanced sample sizes, we could not explore whether the sexes differed in their response to our treatment. Previous work on rodents indicates that sexes may differ in their response (e.g. [16,19]), and with sexual differences in levels of contribution to various prosocial behaviours in meerkats (e.g. [21,24,33]) we may expect to find similar differences when a larger and more balanced sample is considered.

Increasing prosocial behaviours also imposes direct costs on the practitioners as they spent less time foraging, and consequently found less food. Furthermore, of the food that prosocial individuals did find, a greater proportion of it was fed to pups rather than eaten. Adult mass determines propensity to pup-feed and babysit [33,34], and in the case of reproductive females it determines their litter size and pup mass [3]. Therefore, lighter individuals may suffer in both their direct and indirect reproductive output over the long term because of their practice of prosocial behaviours. In addition to costs of reduced food intake, vigorous digging appears to be energetically costly. Costs may also accrue to individuals refraining from initiating aggressive interactions, which are crucial in determining dominance status and hence reproductive success [35].

The simultaneous effect of administering OT across a wide suite of prosocial behaviours means that the trade-off between the benefits (direct and indirect) accruing to the practitioner and the costs that they impose is complex. The coordinated effect of peripherally administered OT across behaviours makes it difficult for the practitioner to decouple behaviours that bring it direct

Figure 2. Individuals’ foraging and excreting behaviours following injection with OT and a saline control: (a) foraging and (b) food eaten. Mean proportions of each activity compared with those of the saline control are shown ±1 s.e.
benefits (e.g. digging shelter burrows) from those that impose direct costs in another form (e.g. feeding an increased proportion of food found to pups). In addition, the practitioner becomes susceptible to exploitation, being unable to decouple prosocial behaviours that produce direct benefits for themselves from behaviours that produce indirect benefits in the form of common goods for other group members while imposing direct costs on the practitioner.

Adult propensity to engage in prosocial behaviours governed by OT can be affected by early life experiences. Although variation in the amount or distribution of OT receptors has a strong genetic component [36], it is also affected, at least in rodents and humans, by early experiences, specifically establishment of social bonds [37–39]. In meerkats, the susceptibility of an individual to the influence of OT could be influenced by the behaviour of other group members, as well as parents. Adults of both sexes contribute to babysitting of pups, during which time they engage in frequent tactile care [34]. If a similar mechanism influencing susceptibility to OT exists in meerkats, as is seen in rodents, then an increased investment in care of pups by helpers would have effects on the organization of OT receptors in the pup’s brain, making them more susceptible to the effects of OT as adults, and so provide these helpers with future benefits. When the pup grows up and becomes a fellow group member, it is more likely to exhibit elevated levels of prosocial behaviours owing to enhanced susceptibility to OT, benefiting other group members both directly and indirectly. This may explain why males who have more adult helpers caring for them perform higher levels of pup feeding as adults [40].

A suite of cooperative and prosocial behaviours in meerkats were modulated by peripheral administration of OT. If this peripheral administration operates in a similar way to the natural OT system, where centrally released OT acts on receptors in specific brain regions, then this finding could provide an explanation for previous studies that show a positive relationship between the expression of multiple cooperative behaviours [4,5]. It may help explain why individuals practise costly behaviours if a single mechanism promotes a mixture of individually beneficial and costly behaviours, but natural selection has not yet managed to decouple components of such a syndrome. Finally, it may explain aspects of heritability of cooperative behaviour in species with tactile parental care, where physiological changes initiated by (allo)parental care carry over to determine adult behaviour.

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