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SHORT COMMUNICATION

Effect of giving birth on the cortisol level in a bonobo groups' (*Pan paniscus*) saliva

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Abstract This study documents the cortisol levels in the saliva of a bonobo group, especially that of a bonobo female which had given birth for the first time. During a long study in Zoological Garden Frankfurt, Germany, a bonobo baby was born on 3 August 2007. Due to the fission–fusion keeping system employed, the bonobos were divided into two groups on this day. Their behavior was observed regularly and saliva was also collected. The bonobos had been trained to chew cotton wool and to give back the samples. The cortisol response was tested twice a day before birth and three times on the day of parturition. Before birth, no observable indication behavior was seen, but an increase in the cortisol concentration of the expectant mother was found. Parturition occurred at 8 pm. The next morning, the group with the newborn was visibly more active, which correlated with the fact that their cortisol levels were increased in the morning in comparison to the second group. During the day, cortisol decreased in both groups, only it was higher throughout the day in the new mother. In the evening, the two groups showed nearly the same cortisol levels. These data indicate that there is indeed a relation between observable behavior and the cortisol level in bonobo saliva. Therefore, the cortisol level can be regarded as a suitable indicator for verifying behavioral events.

Keywords Bonobo · Behavior · Zoo biology · Cortisol · Saliva

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Introduction

Primate parturition has already been documented in the literature on captive nonhuman primates (Brand and Mitchell 1971), as well as in the literature on nonhuman primates in naturalistic environments (Stewart 1977; Goodall 1980). Furthermore, observations were made during the births of bonobos (Hill 1968, Kirchshofer 1962). However, this observational data was not verified by physiological data in any of these studies. We wanted to know whether parturition has an effect on the cortisol levels of members of the whole bonobo group and thus on their behavior.

Many non-invasive methods are known to measure primate "stress" hormones in urine (Layne et al. 1964; Czekala et al. 1994; Bahr et al. 1998; Bahr et al. 2000; Anestis and Bribiescas 2004; Muller and Wrangham 2004), in feces (Whitten et al. 1998; Bahr et al. 2000; Hill et al. 2005; Peel et al. 2005) or in saliva (Lutz et al. 2000; Elder and Menzel 2001). These non-invasive methods of collecting cortisol samples are preferred to blood samples as the latter increase cortisol levels (Francis et al. 1987; Creel 2001). Saliva collection has already been used in a few species: dogs (Beerda et al. 1996), black rhinoceros (Czekala and Callison 1996), and western lowland gorillas (Kuhar et al. 2005), as well as in humans (Francis et al. 1987).

A circadian rhythm in the cortisol level was found in humans (Perkoff et al. 1959; Kirschbaumer and

Hellhammer 1989; Czekala et al. 1994). A diurnal rhythm was also reported for rhesus monkeys (Leshner et al. 1978), chimpanzees (Anestis and Bribiescas 2004), gorillas (Czekala et al. 1994) and orang-utans (Elder and Menzel 2001). In our present study, we determined cortisol levels from saliva as a measure of stress. Due to the circadian influence saliva, was collected twice a day to measure the effects of the event itself and not the circadian rhythm.

Methods

The subjects of the study were nine bonobos (*Pan paniscus*) kept in Zoological Garden Frankfurt, Germany, in a fission–fusion system. The bonobo exhibits were three indoor and four outdoor enclosures. The total area outside was 85 m^2 and 69 m^2 inside. The individual groups changed every few days. On the day of the birth, the group around the mother consisted of the adult male, two adult females and a female infant. The other group comprised two females and two male infants.

During positive reinforcement training, the bonobos were trained to chew cotton wool and to give the samples to the experimenter. On "normal" days, saliva was sampled twice, at 1:00 pm and 4:00 pm, using Salivette R (Sarstedt, Nümbrecht, Germany). On the day after the birth, saliva was sampled three times: at 8:30 am, 1:00 pm and also 4:00 pm. Samples were taken from the bonobo mother, as well as from individuals housed together with the mother and a control group separated from the mother. The salivary sampling device contains a neutral cotton swab and was spiked with some sugar solution to increase the acceptance and the salivary flow of the sampled animals. After saliva collection, the samples were stored at −20°C until analysis. The sampling devices used for collection were centrifuged (3,600 rpm, 10 min) for analysis, and 3 µl of saliva were used without further extraction.

The assay used was described by Palme and Möstl (1997). In brief, the antibody was raised in rabbits against cortisol-3-CMO; BSA and cortisol-3-CMO linked to diamino-3,6-dioxaoctane-biotin was used as label. Affinity-purified anti-sheep-IgG was used (1 µg/well) to coat the microtiter plates. The working dilution of the antibody was 1:100,000; for the label it was 1:500,000; while the range of the standard curve was 0.33–80 pg cortisol.

Results

On "routine" days, the "normal" cortisol level in saliva for the whole group was 4.2 ng/ml (max. 5.7 ng/ml, min. 2.4 ng/ml) at 1:00 pm and 3.1 ng/ml (max. 5.1 ng/ml, min. 1.7 ng/ml) at 4:00 pm. In the two months before the baby was born, the mother rested more than other group members (mother: 87.5%, others: 30.4%) and was less active. The mean cortisol concentration in the saliva of the expectant mother was 8.3 ng/ml at 1 pm and 7.4 ng/ml at 4 pm during this phase. The early sample at 8:30 am is only available for the day after the baby was born.

The behavior was also documented for both groups. The group with the newborn was more active (75.0%, mother excluded) than the other group (50.8%) after the day of parturition. The infant and one adult female were extremely interested in the baby. This interest manifested itself in a tendency to perform certain social activities like eating and sleeping in the vicinity of the mother and her newborn, as well as following her whenever she changed her position in the enclosure. The group without the newborn behaved in a similar way during this period to how they behaved on routine days. On the day after the birth, the cortisol level of the early sample showed an average of 14.5 ng/ml (max. 18.3 ng/ml, min. 10.1 ng/ml) in the group with the newborn. At the same time, an average cortisol level of 6.7 ng/ml (max. 9.6 ng/ml, min. 4.0 ng/ml) was found in the neighboring group. For the mother, a cortisol level of 11.9 ng/ml was detected (Fig. 1). The adult male—the father—also seemed to be more agitated (18.0 ng/ml) than the mother.

Interest in the newborn was observed to decrease during the day. The mother merely had to cope with the new circumstances; for example, to carry the baby while she was climbing and handling wood shavings. At 1:00 pm, the group with the newborn still exhibited a higher value (6.1 ng/ml, max. 9.4 ng/ml, min. 5.5 ng/ml) than the group without the newborn (3.6 ng/ml, max. 5.6 ng/ml, min. 2.7 ng/ml). At the same time, the mother's cortisol concentration was 10.1 ng/ml (Fig. 1).

In the evening, the behaviors of both groups were similar. In the last sampling, at 4:00 pm, the group with a

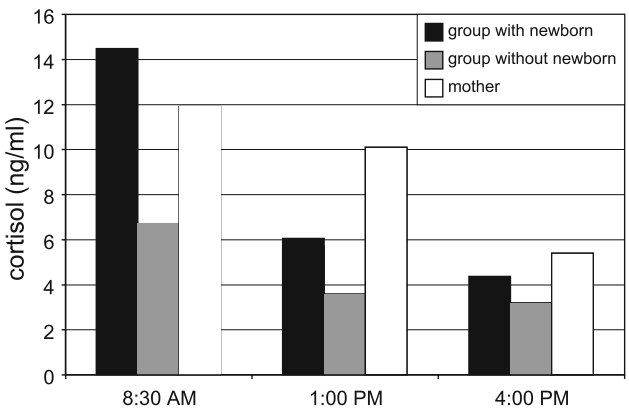

Fig. 1 Mean cortisol concentrations in both groups (excluding the mother of the newborn), as well as the cortisol concentration in the mother

newborn had an average cortisol level of 4.4 ng/ml (max. 6.4 ng/ml, min. 2.4 ng/ml). In the other group, an average of 3.2 ng/ml (max. 4.4 ng/ml, min. 1.2 ng/ml) was found. The cortisol level of the mother was 5.4 ng/ml (Fig. 1).

Discussion

From this study, we can conclude that the cortisol concentration in bonobo saliva is a good indicator for verifying behavioral events.

Similar to our study, Stewart (1977) found no observable indication before parturition. However, one month before she gave birth, the cortisol level of the mother increased in comparison to the level on "normal" days. A similar increase was also found in expectant gorilla mothers (Bahr et al. 1998).

The increased activity in the group with the newborn was linked to an extremely high interest in the newborn; the group members liked to look at or touch the newborn. This kind of interest was also seen in subadult gorillas (Stewart 1977) and adolescent and juvenile chimpanzees (Goodall and Athumani 1980).

The difference between the two groups, however, results from the occurrence of the birth event in the group with the newborn. The decrease in the average cortisol concentration on normal days as well as on the day after the birth is typical. Anestis and Bribiescas (2004) found that there is a diurnal cortisol rhythm in chimpanzees, with the level decreasing throughout the day. Furthermore, high average cortisol levels in the morning were reported by Czekala et al. (1994) for gorillas. This cortisol release is normal for diurnal primates according to Elder and Menzel (2001).

At 1:00 pm, the level of cortisol in the mother's saliva was nearly twice as high as the levels in the saliva of the other bonobos. Kirschbaumer and Hellhammer (1989) documented stable salivary cortisol levels in the early morning as well as a strong influence by internal stimulation. Our data could be interpreted as indicating that the other group members went back to their daily routine, whereas the mother had to cope with the new circumstances, which could be stressful for her.

The diurnal decrease was verified in our study. In the group without the newborn, cortisol saliva averages were almost the same as "normal" cortisol levels. Furthermore, by the end of the day, the cortisol level of the group with the newborn decreases to a level comparable with that of the group without the newborn, with the exception being the mother.

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