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Applied Animal Behaviour Science 93 (2005) 39–52
APPLIED ANIMAL BEHAVIOUR SCIENCE
www.elsevier.com/locate/applanim

Effects of brief maternal separation in kids on neurohormonal and electroencephalographic parameters

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Accepted 12 December 2004
Available online 1 February 2005

Abstract

In goat a selective and reciprocal mother–young bond is rapidly established at birth and persists even beyond the lactation period under natural conditions. Therefore physical separation of mother and young may result in psychobiological disturbance. Aim of this study was to assess quantitative electroencephalography (q-EEG) and neurohormonal markers accompanied by behavioural observations (vocal rate) before and after separation in growing kids. Seven healthy Saanen kids showing normal behavioural patterns were selected just after 2 days from birth. Kids were tested before (basal condition: B) and after separation (separation condition: S) from their mother, respectively at 15 (T1), 30 (T2), 45 (T3), 60 (T4), and 75 (T5) days of age. For each experimental session tests were sequenced, basal test followed by separation test. All experimental trials were videotaped and analysed. Each EEG recording session lasted 10 min. At the end a standardized method for blood collection was implemented. Blood samples were collected in order to perform cortisol serum and catecholamine (epinephrine, norepinephrine, dopamine) plasma levels by radioimmunoassays. q-EEG analysis was performed using Fast Fourier Transform (FFT); the spectral bands delta (0.5–4.0 Hz), theta (4.1–8.0 Hz), alpha (8.1–12.0 Hz), and beta (12.1–30.0 Hz) were calculated and expressed as relative power (%). Kolmogorov–Smirnov test for normality and paired *t*-test were used for statistical comparison. Statistical analysis showed significant increases in cortisol, epinephrine,

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0168-1591/$ – see front matter
doi:10.1016/j.applanim.2004.12.002

norepinephrine levels, and significant decrease in dopamine levels between BT1 versus ST1. Significant increase was found for theta band between BT2 versus ST2, whereas a significant decrease was found for alpha band between BT5 versus ST5. Kids tended to vocalize more in S, but this difference was significant only between BT1 versus ST1 and BT2 versus ST2 (Wilcoxon's test). These results, as well as behavioural data, suggest a main response of both sympatho–adrenal axis and adrenal cortex and a bioelectrical activity response to maternal separation.

Keywords: Maternal separation; Goats; Sympatho–adrenal axis; Electroencephalography

1. Introduction

Studies in young animals have shown that brief separation from mother or siblings results in an increase of vocalization and in behavioural arousal, suggesting psychological stress (Panksepp et al., 1978).

As reported by Hennessy (1997) "separation of partners exhibiting signs of emotional attachment leads to an immediate and persistent hypothalamic–pituitary–adrenal responses (HPA), whereas separation of partners that are affiliative, but do not exhibit attachment, has little or no effect on HPA activity". About goats, it has been reported that the immediate separation after parturition of mother and offspring even for a brief period causes clear disturbances, sometimes irredeemable for the formation of the maternal–filial bond (Ramírez et al., 1996). Indeed in goats a selective and reciprocal mother–young bond is rapidly established at birth and persists even beyond the lactation period under natural conditions (Lickliter, 1982; Addae et al., 2000). Therefore physical separation of mother and young may result in psychobiological disturbance.

Neonatal isolation has immediate effects on various stress–responsive systems. The HPA is activated, resulting in an increased concentration of plasma catecholamines and cortisol, which are essential components of adaptation to stress. An active response to stress is thought to be associated mainly with the activation of the adrenal medulla and the sympathetic nervous system (catecholamines), whereas a passive response is thought to be related to stimulation of the pituitary–adreno–cortical system (Henry and Stephens, 1977). Consistent with growing evidence, stress and the concomitant secretion of glucocorticoids facilitates dopaminergic activity (Piazza and Le Moal, 1998; Piazza et al., 1996). Neonatal isolation also produces immediate changes in brain mesolimbic dopamine systems, consisting in greater dopaminergic turnover in the hypothalamus, septum, and striatum (Kehoe et al., 1997). Experiments showed that the emotional state is accompanied in humans and animals by variations of the electroencephalogram (EEG) (Yamamoto, 1998; Jongman et al., 2000; Aftanas et al., 2002). In particular, in the human EEG research it was suggested that alpha band suppression reflects attentional processes (Klimesch et al., 1998) and theta band power increases with increasing task demands and it is related to orienting (Dietl et al., 1999), attention (Basar et al., 2001), memory (Klimesch, 1999), and affective processing mechanisms (Aftanas et al., 2001).

Aim of this study was to assess quantitative electroencephalography (q-EEG), cortisol serum, and catecholamines (epinephrine, E; norepinephrine, NE; dopamine, DA) plasma

levels accompanied by behavioural observations (vocal rate) before and after brief maternal separation in growing kids in order to investigate whether physical separation of mother and young may result in psychobiological disturbance.

2. Materials and methods

2.1. Experimental site and system of management

This study was carried out at CISRA (Centro Interdipartimentale Servizio Ricoveri) of the Faculty of Veterinary Medicine, University of Turin (Italy). The experiment was conducted during the period from May to July 2002. The mean temperature during the experiment was 22 °C (range 15–28 °C). The C.I.S.R.A. has 20 paddocks ranging in size from 1000 to 2000 m^2. The herd at the C.I.S.R.A. are rotated on these paddocks containing grass forage. In addition, indoor-pens are available and they house the herd at night and during bad weather. After kidding, dams and their kids were housed in individual pens (1 m × 1.8 m) and they were allowed to stay together for 6 h before the kids were weighed, sexed, and ear-tagged. This period of non-interference was necessary to allow sufficient time for dams and their kids to establish a bond. Kids and their dams remained in the individual pens for 6 days to strengthen their bond; after that period does and kids were regrouped with the herd.

2.2. Animals

Seven Saanen single-born kids were studied at the indoor-pen. They were born from multiparous dams. The mean birth weight of the kids was 2.18 kg (range 1.5–3.3 kg); six were females and one was male. Growth and behaviour of the kids were normal. Since the kids were allowed to spend all the time with their dams except for experimental sessions, they were not force-weaned. In addition, growing kids were allowed dams' feed that consists in a mixture of chaff and a commercial stud-goat ration. Water was given ad libitum.

In the week before the experimental session, kids and dams were separated twice a day to familiarise the animals with the procedure. In addition, since we did not use implanted EEG electrodes, kids were submitted to a preliminary electrode placement session to order to familiarise with the practice.

During the experimental sessions the kids were housed in a wire mesh division (100 cm × 53 cm × 100 cm). The EEG recording equipment was housed adjacent to the wire mesh division and the kids were only disturbed if they chewed on the recording leads.

2.3. Experimental set up

Experimental sessions were performed at the indoor-pen. Kids were tested before (basal condition: B) and after separation (separation condition: S) from their mother, respectively at 15 (T1), 30 (T2), 45 (T3), 60 (T4) and 75 (T5) days of age. In condition B, the kid could maintain visual contact with its dam and co-specifics and establish physical contact

(sniffing and licking) with it. In condition S, the kid was physically and visually separated from its dam, but could maintain visual and physical contact with its co-specific pen-mates. For each experimental session tests were sequenced, basal test followed by separation test.

All the experimental sessions were video recorded (Sony DSR-PD1P Camcord Digital Duncam).

2.4. Electroencephalographic recordings

After housing the kid in the wire mesh division, a five channels monopolar montage (F3, F4, Cz, O1, O2; sensitivity = 5 μV/mm; time constant = 0.3 s; Hf = 50 Hz; notch and muscular filters inserted; reference: on the bridge of the nose; ground: caudally to the external occipital protuberance) was used to record bioelectrical activity. Intra-muscular lidocaine injections were not used. Seven EEG needles (30 gauge 15 mm monopolar stainless steel needle electrodes, BIONEN S.A.S.) were used as subdermal active, reference, and ground electrodes. A method of standardized placement of EEG electrodes (Fig. 1), similar to the 10–20 international system used for humans, was used as described by Bergamasco et al. (2003). ECG and respiratory rate were recorded via polygraphic electrodes (X1: sensitivity = 70 μV/mm, time constant = 0.1 s, Hf = 30 Hz; X2: sensitivity = 20 μV/mm, time constant = 0.3 s, Hf = 30 Hz) connected to alligator clips (thin cable for bridge electrode, BIONEN S.A.S.) and a volumetric transducer applied to the chest (thoracic respiratory transducer, BIONEN S.A.S.). Five minutes after the first

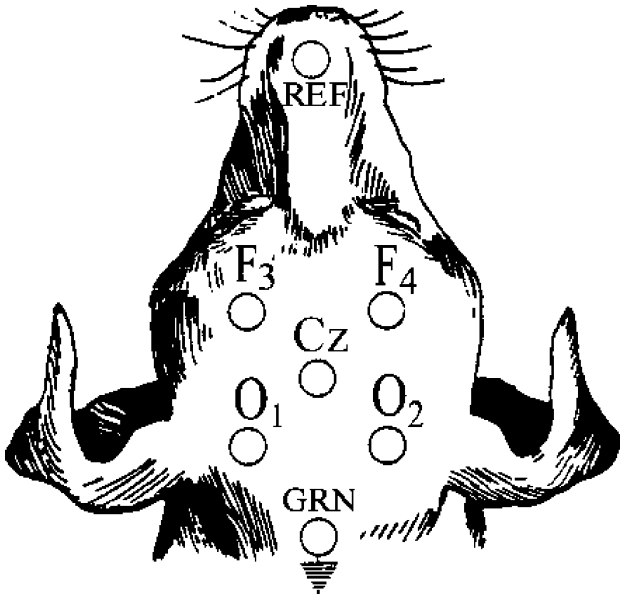

Fig. 1. Montage used in this study. Numbers indicate the hemispheric site; even numbers: right hemisphere; odd numbers: left hemisphere; F: frontal; O: occipital; Cz: medial sagittal line electrode (vertex); REF: reference electrode; GRN: ground electrode.

handling of the kids (specifically after housing the kid in the wire mesh division and placing the electrodes), the EEG recording was started and continued for 10 min for each test. Then EEG data were stored in the acquisition station (Halley Galileo System) for later analyses.

2.5. EEG visual examination

Before performing the quantitative analysis of the bioelectrical activity, visual examination of the EEG traces was done on all the kids. Special emphasis has been placed on artefact detection and elimination, because they strongly affect frequency analysis of the EEG (q-EEG). Ocular movements, cardiovascular, and muscular activity, physiological rhythmic movements or artifacts depending on recording environment (i.e. electrical interference of the powered devices connected to the animals, movement of personnel, acoustic interference) can lead to an incorrect interpretation of the quantitative analysis.

2.6. EEG quantitative analysis

Bioelectrical activity was analysed by a server (RST Galileo System) using an integrated software programme (Fast Fourier Transform). Seven replications of 150, artefact-free 2-s epochs were selected for each experimental condition at different scheduled times. FFT was calculated for each channel and averaged. The spectral bands of delta (0.5–4.0 Hz), theta (4.1–8.0 Hz), alpha (8.1–12.0 Hz) and beta (12.1–30.0 Hz) were calculated. To standardize absolute power across animals, relative power (%) was used in the statistical analysis.

2.7. Blood sampling, plasma catecholamines and cortisol assay

Immediately after each test (i.e. basal test and separation test) blood samples were collected, stored and kept in dry ice until analyses were conducted. Blood sample were taken by jugular venepuncture; the average duration of the sampling was 40 s. The kids generally stood quietly during sampling.

Samples used for determination of plasma catecholamine levels (ng/ml) were collected in 5 ml tubes containing ethylene diaminotetraacetic acid. Plasma was immediately separated by refrigerated centrifugation ($2500 \times g$ for 5 min at 4 °C) then stored at −80 °C. Norepinephrine, E, and DA levels were measured using a commercial radioimmunoassay kit (TriCat^{TM} RIA). The intra-assay and inter-assay coefficient of variation were $4.2 \pm 1\%$ and $5.5 \pm 0.9\%$ respectively, whereas recovery was $92 \pm 2\%$ for NE, $86 \pm 4\%$ for E, and $95 \pm 1\%$ for DA.

Blood samples for cortisol concentration measurements (ng/ml) were collected in 10 ml tubes and allowed to clot at room temperature; then they were centrifuged ($2500 \times g$ for 5 min at 20 °C) and stored at −80 °C until assay. A commercial cortisol radioimmunoassay kit (Cortisol, Immunotech, Pantec, Torino, Italy) was used to measure serum cortisol levels. The intra-assay and inter-assay coefficient of variation were $6.1 \pm 1.3\%$ and $8 \pm 1.6\%$, respectively. The recovery was $95 \pm 4\%$. The samples have been analysed in duplicated measurements and have been calculated using a computer process data analysis (Cubic spline program) as an automated method.

2.8. Behavioural measurements

Vocalizations occurring throughout the experimental sessions were recorded by Sony TCD-D7 Digital Audio Tape-Corder and were analysed as rate of bleating for a 10-min period, corresponding to the duration of the EEG recording sessions. Later on, vocalizations were stored on a CD-ROM as digital wave format. Vocal rates were calculated using an integrated software programme (Voxys 5.0).

2.9. Statistical analysis

Kolmogorov–Smirnov test for normality was employed to check the gaussian distribution of q-EEG and biochemical peripheral data. One-way ANOVA in a repeated measure was first applied to basal and separation conditions at different scheduled times; statistical differences between the two conditions at different scheduled times were then evaluated by paired *t*-test (Graph Pad InStat 3). In our one-way ANOVA experimental design, the selected variable, namely the separation condition, is only related to the age to decrease the effect of confounding variables. Then the selected variable was tested carefully at each scheduled time by using two-tails paired Student's *t*-test. As a result, each mean value was tested only one time.

Friedman test and Wilcoxon's test were used for behavioural data (Graph Pad InStat 3). The probability value was set at $p < 0.05$. Results were expressed as mean ± S.E.M.

3. Results

ECG and respiratory rate recorded during the experimental sessions remained within normal ranges (Mir et al., 2000).

All results of plasma catecholamines and cortisol measurements of basal and separation conditions at the different scheduled time are summarized in Fig. 2.

One-way ANOVA for repeated measures showed significant differences for epinephrine, norepinephrine plasma levels, and cortisol serum levels in both basal and separation conditions (basal E, $p = 0.043$; separation E, $p = 0.0039$; basal NE, $p = 0.0135$; separation NE, $p = 0.0015$; basal cortisol, $p = 0.0092$; separation cortisol, $p < 0.0001$).

Paired *t*-test showed significant increases in E and NE plasma levels between BT1 versus ST1 ($p = 0.028$ and 0.0032, respectively).

Paired *t*-test showed significant increase in cortisol serum levels between BT1 versus ST1 ($p = 0.0018$).

One-way ANOVA for repeated measures showed no significant variations in dopamine plasma levels in basal condition, whereas significant differences were reported in separation condition ($p = 0.035$). Paired *t*-test showed a significant decrease in DA plasma levels between BT1 versus ST1 ($p = 0.0078$).

The preliminary visual examination of EEG recordings from the kids revealed a high voltage–low frequency background activity. Paroxysmal activity or EEG burst-suppression was not observed.

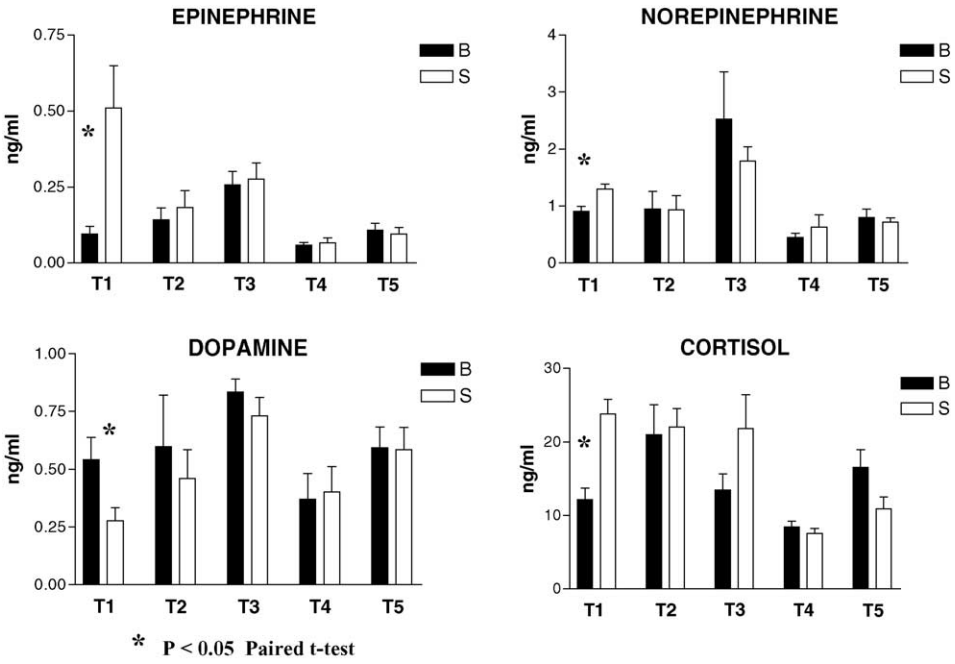

Fig. 2. Catecholamines and cortisol measurements (mean ± S.E.M.) in the two conditions (B: basal; S: separation) at different scheduled times. $^{*}p < 0.05$ paired *t*-test. Results of one-way ANOVA in repeated measures are reported in the text.

Results of the q-EEG findings of basal and separation conditions at the different scheduled time are summarized in Fig. 3. Both conditions showed a prevalence of slow rhythms delta (0.5–4.0 Hz) and theta (4.1–8.0 Hz), while fast rhythms alpha (8.1–12.0 Hz) and beta (12.1–30.0 Hz) were poorly represented.

One-way ANOVA for repeated measures showed no significant variations in relative powers of delta band in basal condition, while significant differences were reported in separation condition ($p = 0.0394$).

One-way ANOVA for repeated measures showed significant variations in relative powers of theta and beta bands in both conditions (basal theta, $p = 0.0284$; separation theta, $p = 0.0142$; basal beta, $p = 0.0424$; separation beta, $p = 0.0001$). Paired *t*-test showed a significant increase of theta band between BT2 versus ST2 ($p = 0.0268$).

One-way ANOVA for repeated measures showed no significant variations in relative powers of alpha band in both conditions. Paired *t*-test showed a significant decrease was found for alpha band between BT5 versus ST5 ($p = 0.0399$).

Results of the vocal rate (number of vocalizations) of basal and separation conditions at the different scheduled time are summarized in Fig. 4.

Friedman test showed no significant variations in basal vocal rates, whereas separation vocal rates showed significant differences ($p = 0.001$). Significant increases of vocalizations were found between BT1 versus ST1 ($p = 0.0156$) and between BT2 versus ST2 ($p = 0.0156$) by Wilcoxon's test.

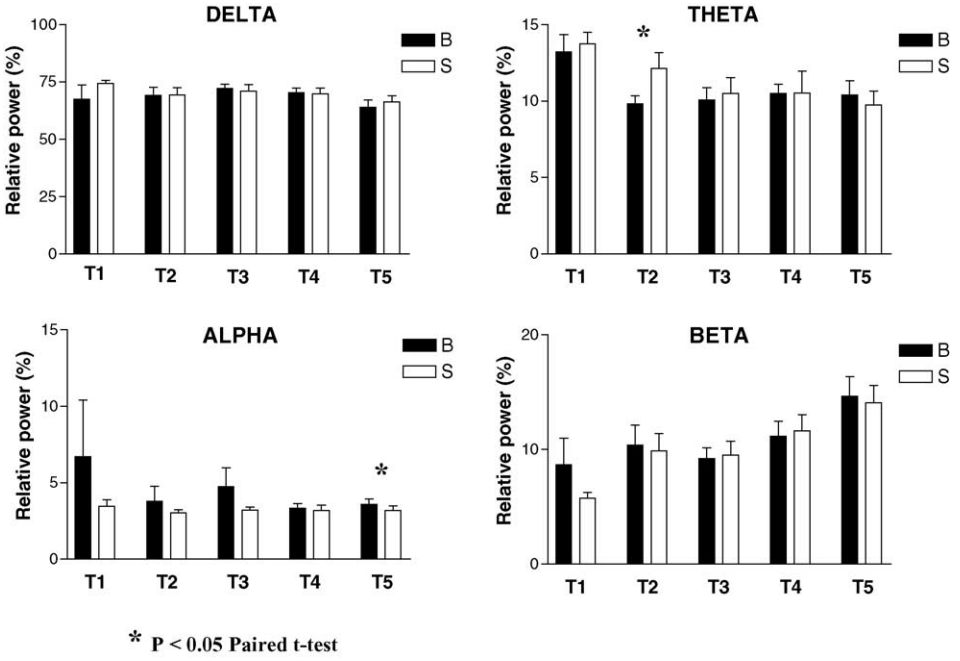

Fig. 3. Relative (%) power data (mean ± S.E.M.) recorded from all the electrodes of the four frequency bands (delta, theta, alpha, beta) in the two conditions (B: basal; S: separation) at different scheduled times. $^{*}p < 0.05$ paired *t*-test. Results of one-way ANOVA in repeated measures are reported in the text.

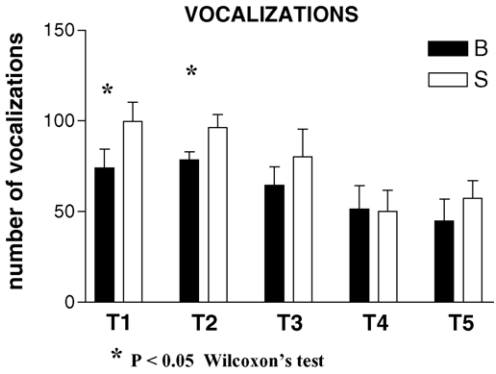

Fig. 4. Vocal rate (mean ± S.E.M.) in the two conditions (B: basal; S: separation) at different scheduled times. $^{*}p < 0.05$ Wicoxon's test. Results of Friedman test are reported in the text.

4. Discussion

In goats, mothers rapidly form a bond with their offspring during the postpartum period. Kids display a preference for proximity to the dam rather than an alien female in less than 48 h of age (Lickliter and Heron, 1984a), but the manifestation of mother–young bonding

persists after the sensitive period. Studies in barn conditions suggest that it is mainly the mother that is responsible for the initiation and maintenance of contact during the first week of the kid's life, but afterwards this role is adopted by the young (Lickliter, 1984b).

Independent of the early environment, it has been shown that the behavioural and physiological responses to brief maternal separation are not confined to primates and rodents (Berman et al., 1994; Laudenslager et al., 1995; McCormick et al., 1998), in which filial attachment is most evident (Hennessy, 1997), but they are reported in goats as well (Lickliter and Heron, 1984a). The initial behavioural reactions are restless, increased locomotion and distress vocalizations, while physiological stress responses mainly result in HPA activation.

At the neurobiological level, maternal separation in juvenile primates induces increased cerebrospinalfluid (CSF) norepinephrine levels (Kraemer et al., 1991) and PET scans have identified altered cortical activation (Rilling et al., 2001).

The sudden unexpected absence of the mother can be classified as a psychogenic stressor (Hennessy, 1997). In addition, such separation exemplifies aspects of other psychogenic stimuli to which the HPA axis is particularly responsive, namely, novelty, uncertainty and loss of control. Especially, uncertainty and anxiety are among the most potent stimuli for HPA activation and sympatho–adrenal medullary axis, resulting in an increased concentration of plasma catecholamines and cortisol (Sconberg et al., 1993), which are essential components of adaptation to stress. As concern stress-induced variations of catecholamine and cortisol plasma levels in goats, it is reported (New et al., 1996) that transportation stress activated the HPA and the sympatho–adrenal medullary axis. Especially, both plasma and salivary cortisol levels are useful measures of adrenocortical response to stress in kids and adult goats (Greenwood and Shutt, 1992). However, detailed information concerning stress in these animals is scarce. In addition, changes in catecholamines and cortisol levels in growing kids exposed to maternal separation have not been reported.

In our study, brief maternal separation (10 min) produced significant increases in both behavioural and physiological measures, suggesting that maternal separation could be an effective psychogenic stressor in growing kids.

Kids responses to brief separation from their mother and co-specifics pen-mates were investigated by Lyons et al. (1988). Nevertheless, because of the aim of the above mentioned study, the results about corticosteroid levels revealed as much about the presence of a human in the pen rather than the response to maternal separation.

Our results reported a significant increase in E, NE, and cortisol levels of 15-day-old kids after maternal separation, whereas in older kids no significant variations were reported. However, increased values of E and cortisol were reported in separation condition up to T3 (45 days of age). This fact could indicate that a simultaneous activation of adrenal medulla and cortex has occurred resulting in the so-called mixed response to stress that involves HPA and sympatho–adrenal medullary axis activation (Mitchell et al., 1988).

As concern DA plasma levels, a significant decrease was reported for 15-day-old kids after maternal separation, whilst in older kids no significant variations were reported. However, DA decreased values were reported in separation condition up to T3 (45 days of age).

The role of DA as neurotransmitter or neuroendocrine modulator has been reported in the central and peripheral nervous system of humans and other mammals (Bell and

O'Connell, 1999; Hadjiconstantinou and Neff, 1987; Lackovic and Relja, 1983; Velasco and Luchsinger, 1998; Yoshizumi et al., 1995). In the central nervous system, DA tonic inhibitory effects occur in several brain functions involved in the emotional behaviour and reproduction (Bell and Hepper, 1987; Sharp et al., 1984). Neonatal isolation produces immediate changes in brain mesolimbic DA system (Kehoe et al., 1997) as well as acute restraint stress has been reported to decrease dopamine synthesis and turnover in the median eminence (Demarest et al., 1985; Gala, 1990). The biphasic alteration of DA transmission in the mesolimbic system (i.e. rising and falling) could suggest that the initial increase of DA release represents an arousal response, while the subsequent decrease in DA release may be related to coping failure (Puglisi-Allegra et al., 1991). Whether the variations in DA plasma levels reported in the present study reflect similar changes in brain DA is still unknown, since brain and blood DA are release from different sources, and systemically circulating DA cannot cross the brain–blood barrier. However, recent studies have shown that both brain and plasma DA pools might be interrelated. Friedhoff and Simkowitz (1989) reported that the brain makes a large contribution to the plasma DA metabolites pool, since neuroleptics similarly affect DA release from brain and peripheral dopaminergic system. However, further studies are needed to determine whether similar regulations of blood and brain DA are present in goats.

Nowak (1996) suggests that vocal communication between the ewe and the lamb is central to adequate bond formation, and has demonstrated that lamb bleating behaviour is involved in the attachment process (Nowak, 1990). Vocalization can be influenced by general excitation or arousal that occur during maternal separation; conversely, it can be considered as a coping response that acts to reduce arousal and associated elevations in HPA activity (Levine et al., 1984). Results from the present study report a significant increase of vocal rate in separation condition at T1 and T2; these data can be related to the increase of NE, E, and cortisol levels observed in the same scheduled time, reflecting the general excitation or arousal that occurred during maternal separation.

Therefore, neurohormonal levels as well as behavioural data suggest a main response of symphato-adrenal axis (Minton, 1994) and adrenal cortex (Sanhouri et al., 1989) to maternal separation. The bioelectrical activity response, namely the increase of theta frequency band could be related to the emotional state of the kids in separation condition.

In a physiological sense, EEG power reflects the number of neurons that discharge synchronously and it can be tempting to assume that EEG power is a measure that reflects the capacity or performance of cortical processing information. In addition, relative power measurements tend to give lager estimates for the dominant frequency range (where absolute power is largest) and lower estimates for frequencies, which fall outside this range.

Animal research has shown that theta is an oscillatory component of the hippocampal EEG, which is related to memory process (Miller, 1991). In fact, from an anatomical point of view, the hippocampus belongs to the limbic system but it is also a component of the Papez circuit, which is related to emotionality and memory, respectively.

Theta frequency ranges from about 3 to 12 Hz (Lopes da Silva, 1992) and its great power makes it easy to observe frequency and power changes in animals. In our study, we set theta and alpha bandwidth as reported by Jongman et al. (2000).

Theta and alpha oscillation defined in narrow frequency bands are regarded as reflecting activity of multifunctional neuronal networks, differentially associated with sensory, cognitive, and affective processing (Basar et al., 2001). Conversely, alpha and theta respond in different and opposite ways. If EEG power in a resting condition is compared with a test condition, alpha power decrease (desynchronizes) and theta power increases (synchronizes).

Many results suggest that higher theta activity is better interpreted as an electrophysiological manifestation of higher activation, related to orienting, attention, memory, affective, and cognitive processing (Aftanas et al., 2001). ''Orienting'' is a coordinated response which appears to indicate alertness, arousal or readiness to process information and it is manifested in cat experiments during exploration, searching and motor behaviour (Basar, 1999). Therefore, orienting is closely linked to attentive state and learning. Since brief separation from the mother results in an increase of behavioural arousal, it could be related to an attentive state that underlies some neurobiological basis of orienting.

Desynchronization in the lower and medium alpha bands is associated with the process of external attention, such as alertness/vigilance and expectancy, whereas desynchronization of upper alpha reflects enhanced cognitive processing (Klimesch et al., 1998).

Results from the present study show a significant increase for theta band between basal and separation condition in 30-day-old kids, whereas a significant decrease was found for alpha band between the two conditions in 75-day-old kids. However, in the present study, alpha band always decreased in separation condition throughout the scheduled times, suggesting a phasic oscillation related to this condition.

According to literature, emotional/affective processing seems to be frequency-dependent, revealing that among the four frequency bands analysed, the theta oscillating networks could be the fastest in discrimination. However, further investigation will be necessary to determine which of the narrow theta and alpha bands are most affected in young small ruminants.

Furthermore, in the present study an increase in faster rhythms as well as a slight decrease in slow frequency bands was noted throughout the scheduled times. This increase in EEG frequency could reflect brain maturation process, consisting in integrations and interconnections of different brain area feedback loops. Nevertheless, data about morphogenesis of the nervous system and histochemical aspect of myelination in kids are not available yet, therefore studies carried out on other ruminants (Takeuchi et al., 1998) have been taken into account. The relative maturity of kids at birth, particularly with respect to coordinated motor activity immediately after birth could suggest a limited postnatal brainstem maturation. Conversely, according to physiological age-dependent changes in responsiveness to visual, auditory and chemosensory cues that augment the efficiency in the learning ability of the kids, it is possible to hypothesize a postnatal maturation of the subcortical and cortical function.

However, we must be caution in comparing results with our isolation paradigm to other paradigms involving separation of offspring from dams. Maternal separation procedures vary in the literature as the length of and the number of episodes of separation and ages at which the separation occurs. There are also important variables such as temperature of the environment in which separated kids are kept, whether the kids are isolated or in proximity

of cues from siblings, and the familiarity of the environment during separation. As a result, maternal isolation involves a cascade of effects, the precise course of which depends on the environmental events that the animal encounters over the life span.

To conclude, in the present study brief maternal separation induces neurohormonal and behavioural changes in 15-day-old kids, whereas changes in the q-EEG occur in older kids. These results might indicate a psychobiological disturbance. Finally, the q-EEG encouraging findings highlight the need to conduct further research to develop this methodology in animal welfare.

Acknowledgements

The authors thank Dr. Jeanne Griffin for linguistic revision. This work was supported by grants from the University of Torino (ex-60%).

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