

Synthetic glucocorticoids, like dexamethasone (DEX), are widely used to prevent chronic lung disease in premature born infants. Notably, high doses of DEX are given during a period of life when the brain is still immature and developing. Although the acute benefit of this treatment, reduced requirement for assisted ventilation, is now well-established, major concern has arisen about the long-term adverse effects of this neonatal DEX therapy [1, 2]. Human follow-up studies are scarce, but those available indicate an increase in the rate of neurodevelopmental impairments in infants of 2-5 years that were treated neonatally with DEX [1, 2]. To investigate possible long-term consequences of neonatal DEX treatment, we developed an animal model using a DEX treatment protocol in rat neonates resembling the one used in clinical practice for preterm infants.

RESEARCH QUESTION

Glucocorticoids affect a number of tissues and thereby influence e.g. mood, behavior, cognition and social behavior [2]. Although humans are social organisms par excellence and inappropriate social interactions with conspecifics may have major impact on daily functioning, nothing is known about the long-term effect of neonatal DEX treatment on social interactions in humans. Therefore we studied the effects of neonatal DEX treatment on social behavior of rats.



METHODS

Rat pups were injected with DEX on neonatal day 1, 2 and 3 (experiment group 'DEX') or received saline (experiment group 'SAL'). To reveal any long-term effects of saline injection, a third group of rats was included which received no treatment (experiment group 'UNTR'). Rats were weaned at day 21 of age and remained group-housed two per cage with same-sex littermates until experimentation. Social play behavior, the first non-mother directed social behavior [4], was tested in juveniles (day 25). Subsequently the rats were left undisturbed until adulthood (day 90) when social activity was tested.

SOCIAL INTERACTIONS IN JUVENILES

The characteristic element of play behavior (or play fighting) is 'pinning'; one of the animals is lying with its dorsal surface on the floor of the test cage with the other animal standing over it [4]. In addition, juveniles also perform social interactions with conspecifics that can be seen in adult rats too, e.g. social exploration and grooming of conspecifics. Therefore, we were interested in a detailed description of the behavioral elements occurring during play behavior and used The Observer to quantify any differences in social interactions (latency, frequency and duration of social behavioral elements) between DEX and SAL juveniles.

Play behavior was tested by introducing two neonatally similarly treated rats in a test cage for 15 min. Testing was recorded on videotape and behavior was assessed using The Observer (continuous sampling method). Latency, frequency and duration of pinning, social grooming, social exploring, social mounting and following were scored. All the other (non-social) behavioral elements were scored as 'rest'. Data were analyzed with the elementary statistics module of The Observer. Analysis revealed that the frequency and duration of play behavior ('pinning') was enhanced ($P < 0.05$) in DEX as compared to SAL and UNTR rats. However, frequency and duration of social exploration

decreased ($p < 0.05$) in DEX juveniles. There were no differences in total duration of the 'rest' element between groups, indicating that the total duration of social behavior was similar between groups.

ADULT SOCIAL INTERACTION

In adult rats, our main interest was if neonatal DEX would influence the total duration of social interactions. Therefore we used EthoVision, which would give quantitative measures about social activity and interest as described previously [5]. Briefly, experimental adult rats were placed in a circular, familiarized open field (130 cm diameter) for 20 min with an unknown, marked, socially housed stimulus rat. The two rats, discriminated by apparent size (by painting one rat half black, it appeared to be smaller), were tracked with EthoVision. The position of both rats was determined every second. General activity (locomotion) and movements towards (approaching) or from (avoidance) each other were registered. Approach and avoidance behaviors were defined on the basis of the direction of movements of both animals and changes in the inter-individual distance. Both animals could be approaching or avoiding at the same time. EthoVision calculates the relative contribution of each animal to a change in positions. The weight attributed to the direction of movement is related to the inter-individual distance: the closer the two animals are, the more important the direction of movement. The directed movements (approach, avoidance) have been shown to be representative for social activity [5].

It turned out that approach and avoidance behaviors towards the test partner in the social interaction test were similar between groups. Thus no indications were found for altered social interest or social activity in adult rats neonatally treated with DEX. Perhaps if social behavior of the adult rats was videotaped and analyzed in more detail using The Observer (as was done for play behavior in juveniles), differences in particular aspects of social interactions could have been found between DEX and SAL rats. Indeed, the finding that total duration of social behavior during play testing was unaltered, but pinning- and exploration behavior were respectively enhanced and decreased in DEX juveniles supports this notion. Future experiments using The Observer will focus on the intrinsic organization of the social interactions in adult DEX rats.

CONCLUSIONS

Neonatal DEX treatment resulted in enhanced social play (pinning) behavior in juvenile rats. In contrast, social activity during the social interaction test was not altered in adult DEX rats. Thus, it is not social activity per se that is affected in DEX rats, but rather specific aspects of behavioral responses to social challenges. In view of the frequent application of DEX in preterm infants, these data warrant investigation of lasting and potentially adverse effects of treatment of human neonates with DEX on social functioning.

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