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The effect of different housing conditions on behavioural and adrenocortical reactions in veal calves

R. DANTZER, P. MORMÈDE, R. M. BLUTHÉ, J. SOISSONS (*)

Neurobiologie des Comportements, I.N.R.A., Université de Bordeaux II, 146, rue Léo-Saignat, 33076 Bordeaux Cedex, France.

(*) *I.T.E.B. « Monvoisin », Cidex 71 bis, 35650 Le Rheu.*

Summary. Veal calves were housed either tethered indoors on slatted floorings or loose in groups of eight in a semi-open barn with straw bedding.

Six weeks after arrival at the station, the calves were submitted to a 10-min open-field session in either the same environment as the one in which they were housed or in the alternative environment. During this session, activity, latency time to first movement and duration of immobility were scored and blood samples were taken for cortisol determination before and after the session.

In both instances, the tethered calves exhibited higher activity scores and shorter periods of immobility. Plasma cortisol levels were significantly affected by testing conditions but not by housing conditions, *i.e.* plasma cortisol levels increased more when the animals were tested in a different environment from the one in which they were housed. Adrenal cortex response to repeated blood sampling and to ACTH injections was found to be significantly higher in tethered calves.

These results show that housing conditions strongly influenced the behaviour and endocrine activity of the calves. The dynamics of these changes need further study.

Introduction.

Different husbandry systems may be used to house veal calves. Individual housing in crates now tends to be replaced by collective housing on slats or in straw yards, the animals being either tied up by a collar or left to move freely.

There has been much concern about the welfare of calves raised in confinement. Comparisons of calves kept on straw with those raised in crates or kept individually have revealed behavioural differences which have been interpreted as expressions of frustration due to deprivation of basic behavioural needs (Van Putten and Elshof, 1982). However, this interpretation is discutable since most of these differences are related only to the range of available environmental stimuli. Studies in this field have been mainly observational, *i.e.* they have been restricted to a consideration of the spontaneous behaviour of calves under the housing conditions studied with no attempt to assess their reactivity to external stimulation (Ilan *et al.,* 1973 ; Riese *et al.,* 1977 ; Van Putten and Elshof, 1982). Such a test might reveal underlying emotional disorders that would not be apparent when studying the animals in their usual surroundings (Dantzer and Mormède, 1981 ; Moberg and Wood, 1982).

It has been demonstrated in numerous species that low-stimulus input during early life produces anomalous behaviour, but these anomalies are manifested mainly under conditions of excessive or unexpected stimulation when the subjects must adjust quickly (Denenberg, 1969 ; Levine *et al.,* 1967). Simultaneous consideration of physiological functions, such as the reactivity of the pituitary-adrenal axis, can characterize more precisely an animal's ability to cope (Ursin, 1980). For example, rats and mice deprived of infantile stimulation exhibit a lower level of activity and reduced ability to avoid electric shock, together with increased secretion of adrenocortical hormones (Hess *et al.,* 1969 ; Levine *et al.,* 1967).

The purpose of the present study was to assess behavioural and adrenocortical reactions of calves to an open-field test when they had been housed either loose in straw yards (« loose calves ») or tethered on slats (« tied calves »). Their adrenocortical reactivity to an ACTH stimulation test was also examined.

Material and methods.

Animals and housing. — We used commercially bred French Friesian male and female calves approximately 8 weeks old that were brought to the rearing unit 6 weeks before the experiment began.

One group was housed in an air-controlled room separated by a central alley into two pens (12 × 2.34 m), each containing 14 animals. Mean daily temperature and relative humidity were 15.9-20 °C (min-max) and 64-78 %, respectively. The calves were kept on a wooden slatted floor and individually tethered by a chain around the neck. Small wooden boxes (0.57 × 1.60 m) separated adjacent animals.

A second group was housed in an adjacent open shed divided into 8 straw yards (3 × 4 m), each containing 8 calves. The front of the shed was protected from the wind by straw bundles and a removable nylon wind screen.

Both groups were fed a milk replacer (18 % fat and 23 % protein) in individual buckets twice a day, except on Sundays when only one meal was given.

Open-field testing. — Due to different housing conditions, two different environments were used for open-field testing :

1) a 12 × 2.35 m open-field arena with a wooden slatted floor similar to the indoor pens. The floor was marked off into 12 rectangles to estimate ambulation (number of rectangles entered). This arena was evenly illuminated from above by incandescent bulbs ;

2) a 4.70 × 4.50-m open-field arena formed by metal barriers and straw bundles rising from the floor to a height of 1.60 m. The floor was covered with straw and marked off into 4 rectangles to estimate ambulation. This arena, located in the

open-shed part of the building, was not adjacent to the straw yards used for housing the calves.

The observer sitting on a 2-m ladder in a corner of the arena used a data collector (Electro General Datamyte 1000) to record the behavioural patterns displayed by the animals during the test.

The calf to be tested was put on a trolley and carried into the arena where it was released at the beginning of the test. During the 10-min session, latency time to first movement, number of rectangles entered, duration of immobility, vocalization and incidence of stereotyped behaviour were recorded.

Blood sampling and adrenal stimulation test. — The blood of the calf to be tested in the open-field was sampled in the pen just before the animal was carried into the arena and at the end of the open-field trial. Blood samples were drawn by venipuncture from the jugular vein into a 5-ml plastic syringe and immediately transferred into centrifuge tubes containing heparin.

Sixteen different male calves were submitted to an ACTH adrenal stimulation test. They were treated with either ACTH (0.25 mg of Synacthen [R] per animal) or saline injected intravenously immediately after the first blood sampling. Other blood samples were taken 10, 30 and 60 min later.

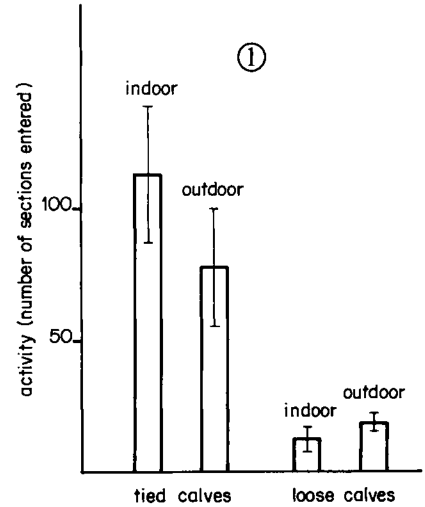
Cortisol analysis. — Following centrifugation of the blood samples, aliquots of plasma were stored at − 20 °C until assayed for cortisol content by the competitive protein binding assay of Murphy (1967). The transcortin source was dog serum (1 %) ; tritiated cortisol (107 Ci/mmol ; CEA, Gif-sur-Yvette, France) was used as a tracer and dextran-coated charcoal as an adsorbent of free radioactivity.

Results.

Eleven calves of both sexes in each of the housing conditions were subjected to the open-field test ; six animals were tested in the indoor arena and five in the open-shed arena. Since there was no behavioural sex difference between the groups, the behavioural results were pooled and submitted to a 2-way analysis of variance (housing conditions × test conditions) with correction for unequal group size.

The ambulation score (fig. 1) was significantly affected by housing conditions ($F(1,18) = 22.6$; $p < 0.001$), tethered animals being more active than loose ones. The total duration of immobility (fig. 2) was significantly affected by both testing ($F(1,18) = 6.98$; $p < 0.05$) and housing ($F(1,18) = 10.1$; $p < 0.01$) conditions ; tethered animals were less immobile than loose animals, and immobility was more pronounced in the indoor arena with slatted floor.

Latency time to first movement tended to be longer in loose calves than in tethered calves (2.03 ± 0.94 min *vs* 0.27 ± 0.14 min ; $F(1,18) = 3.96$; $p < 0.10$). Only one animal from each group vocalized during the test. One other animal in each group licked the walls for a little over 1 min in both cases.



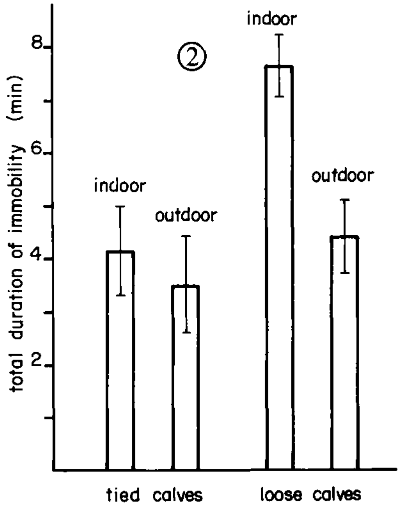
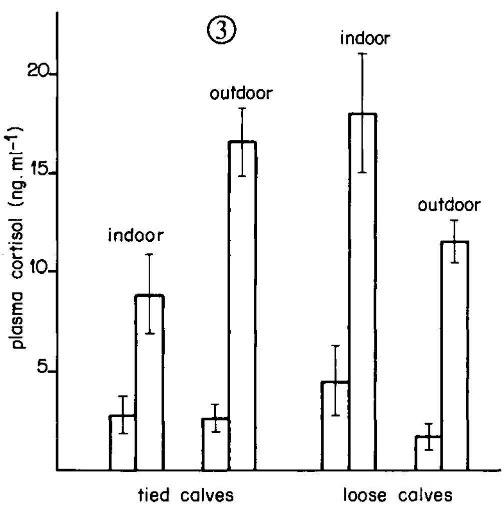


FIG. 1. — *Ambulation score* (mean ± SEM) *of calves raised differentially to the open-field test.* The basis of the test was the type of arena used as an open field.

FIG. 2. — *Total duration of immobility* (mean ± SEM) *of calves raised differentially to the open-field test.* The basis of the test was the type of arena used as an open field.



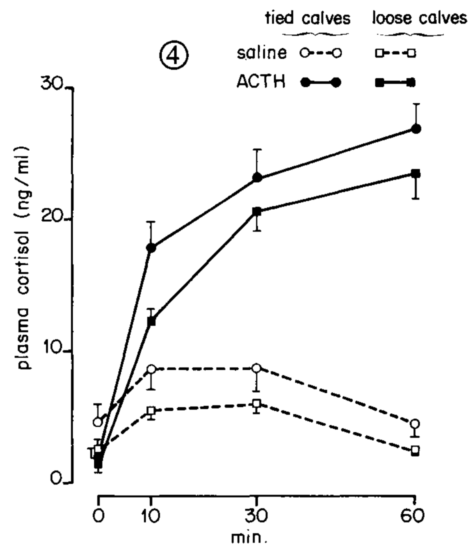


FIG. 3. — *Plasma corticosteroid levels* (mean ± SEM) *of differentially raised calves exposed to different types of open field either indoors or outdoors.* The left column of each group represents the plasma corticosteroid levels in a blood sample just before the open-field session ; the right column represents a second sample drawn just at the end of the open-field session.

FIG. 4. — *Adrenal cortex response* (mean ± SEM ; n = 4) *of differentially raised calves injected intravenously with either ACTH* (0.25 mg) *or saline at time 0.* Blood samples were taken just before injection and 10, 30 and 60 min later.

Plasma cortisol levels before the open-field test were not affected by either housing conditions ($F(1,18) = 0.18$) or the sex of the animal ($F(1,18) = 1.78$). Variations in plasma cortisol (differences between plasma cortisol levels measured after and before the open-field test) were significantly influenced by the interaction of housing test conditions ($F(1,12) = 9.12$; $p < 0.01$), indicating that plasma cortisol levels increased more when calves were tested in a different arena from their housing conditions (fig. 3).

Results of the ACTH stimulation test are presented in figure 4. A 3-way analysis of variance with repeated measurements revealed the significant effect of treatment (ACTH *vs* saline : $F(1,12) = 124.4$; $p < 0.001$), housing conditions (loose *vs* tethered : $F(1,12) = 8.50$; $p < 0.05$), time ($F(3,36) = 9.50$; $p < 0.001$) and treatment $\times$ time interaction ($F(3,36) = 7.58$; $p < 0.001$). Cortisol levels were increased after both ACTH and saline injection, but with a different time-course and intensity. Cortisol levels rose higher after ACTH injection than after saline injection ; they returned to initial values after 60 min in animals injected with saline but remained elevated in animals injected with ACTH. Whatever the treatment, adrenocortical hormone response was greater in tethered calves.

Discussion.

The open-field test, based mainly on the results of experiments in rats and mice (see Archer, 1973), has been used as a standardized stressor in cattle (Kilgour, 1975), sheep (Moberg and Wood, 1982 ; Torres-Hernandez and Hohenboken, 1979), pigs (Dantzer, 1979) and chickens (Jones, 1977 ; Gallup and Suarez, 1980 ; Murphy, 1978). However, with the noticeable exception of chickens, little attempt has been made to assess its validity as a method for measuring fear. It is generally believed that the locomotive activity of an animal in a novel situation is the result of two tendencies : (1) the need to explore and (2) the tendency to freeze in order to avoid predators. However, in many species, escape rather than freezing is the predominant response to fear so that high locomotive activity may express attempts to find a way-out rather than uninhibited exploration. An useful indication may be latency time to first movement and the division of activity within the space of the arena ; high levels of fear are associated with long latency before movement and activity restricted to the outer perimeter of the open-field, the interior of the field being avoided.

In the present study, the size and shape of the yards precluded such differential measurements. In spite of this limitation, it was clear that the increased activity displayed by tied calves could not be ascribed to higher levels of fear, but merely reflected very peculiar behaviour ; in many cases, the animals darted to one side of the yard but slipped to the other side ! This increased activity when compared with calves housed on straw would therefore appear to be a compensatory reaction to deprivation of activity when tethered, possibly potentiated by enhanced responsiveness to the novelty of the test situation. This interpretation is supported by the finding that tethered calves tended to stay immobile for a shorter time in the outdoor arena with straw than in the more familiar indoor arena with slatted floor. This last difference, also apparent in loose calves, probably had a different origin, however, because calves housed on straw were indeed very reluctant to walk on slats.

Interestingly enough, pituitary-adrenal response was differentially affected by the type of environment in which the test took place. Cortisol changes were more marked when the test environment was different from the housing environment. These results are in agreement with the graded sensitivity of the pituitary-adrenal axis to novelty demonstrated by Hennessy and Levine (1978). Increases in cortisol levels induced by the novel situation were not correlated with behavioural reactions since the mean change in cortisol level was the same whatever the rearing experience. This lack of difference is not surprising ; the factor of novelty in first exposure to the open-field situation might overcome the effect of other factors. The same phenomenon has been found in differentially raised rhesus monkeys (Sackett *et al.,* 1973) and lambs (Moberg and Wood, 1982) exposed to novel environments. We have also observed that pigs introduced into a novel environment alone or in pairs displayed the same rise in plasma cortisol in spite of wide behavioural differences (unpublished observations).

ACTH stimulation is a more specific test of the functional state of the adrenal cortex. It reveals differences in the functional activity of the pituitary-adrenal axis which are not apparent or very slight when only basal plasma corticosteroid levels are considered. For example, alteration of the adrenal response to ACTH in the absence of any variation in basal plasma corticosteroid levels was demonstrated by Friend *et al.* (1979) in dairy cows which had to compete for access to a limited number of stalls over several days. In the present study, the ACTH stimulation test demonstrated unequivocally that the adrenals of tied calves were more active than those of loose calves. The pituitary-adrenal axis has been shown to be influenced by the degree of control the animal can exert over its environment (Weiss, 1972). Therefore, we might speculate that the lower degree of initiative allowed to tied calves made their adrenal cortex more reactive than that of their loose congeners. This difference is also apparent in calves subjected to repeated blood sampling, although this procedure in the present study was devoid of any novelty and had little activating effect since our animals were already used to handling for blood sampling, chemotherapeutics and weighing. Neither can possible differences in restraint and handling account for the present data since the reverse trend would be expected if these factors were effective, *i.e.* loose calves which could run away when we were trying to catch them should have had higher cortisol levels.

In conclusion, the present experiment shows that calves tied up on slats reacted to a novel environment by hyperactivity when compared to loose calves kept on straw. Exposure to the novel environment induced a rise in plasma cortisol related to the degree of novelty of the test situation but not to the rearing situation. Adrenal cortex response to repeated blood sampling and ACTH injection, however, was significantly increased in tied calves. The dynamics of behavioural and physiological changes in calves induced by different housing conditions need further study.

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Résumé. *Influence des conditions d'élevage sur la réactivité comportementale et neuro-endocrinienne du veau.*

Des veaux de lait sont élevés soit à l'attache, sur caillebotis, dans un bâtiment conditionné, soit en groupe, sur paille, en semi plein-air. Six semaines après leur entrée en engraissement, ils sont exposés à un environnement nouveau pendant 10 min pour tester leur réactivité comportementale et neuro-endocrinienne. L'environnement nouveau consiste soit en un enclos paillé situé à l'extérieur, soit en une loge avec caillebotis située à l'intérieur. Pendant la durée du test, un observateur enregistre la latence du premier mouvement, l'activité locomotrice et la durée des phases d'immobilité. Des prélèvements sanguins sont effectués avant et après le test pour mesurer les taux de cortisol plasmatique. Quel que soit l'environnement nouveau utilisé, les veaux élevés à l'attache présentent une plus grande activité et une moindre durée des phases d'immobilité. La cortisolémie est accrue après le test, l'augmentation étant d'autant plus importante que le nouvel environnement est plus différent de l'environnement habituel des animaux. L'augmentation de l'activité hypophyso-corticosurrénalienne lors d'exposition à l'environnement nouveau ne dépend pas des conditions d'élevage. Par contre, un test de stimulation surrénalienne par de l'ACTH administré par voie générale, révèle une plus grande activité du cortex surrénalien chez les veaux à l'attache que chez les veaux en groupe.

Ces résultats montrent que les conditions d'élevage modifient la réactivité comportementale et neuro-endocrinienne du veau. Des études complémentaires sur le délai d'apparition et la dynamique de ces altérations sont cependant nécessaires.

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