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Influence of Shock-Induced Fighting and Social Factors on Dopamine Turnover in Cortical and Limbic Areas in the Rat

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DANTZER, R., D. GUILLONEAU, P. MORMÈDE, J. P. HERMAN AND M. LE MOAL. *Influence of shock-induced fighting and social factors on dopamine turnover in cortical and limbic areas in the rat.* PHARMACOL BIOCHEM BEHAV **20**(3) 331–335, 1984.—The present experiments investigated changes in dopaminergic mesocorticolimbic neurones originating from the A10 cell group, in animals exposed to electric shocks in pairs or individually, in comparison to animals receiving no shock and tested in pairs or alone. The social setting under which shock occured had no influence on the increases in DOPAC levels observed in animals exposed acutely or chronically to electric shocks. In contrast, subordinate rats in the paired shock condition had lower tyrosine hydroxylase activity in the accumbens than dominant rats. Pairing of animals in the test cage without shock induced an increase in accumbens DOPAC levels.

Stress Dopamine DOPAC Shock-induced fighting Social factors

RECENT studies suggest that the mesocorticofrontal and the mesolimbic dopaminergic (DA) neurons originating from the A10 cell group located within the ventral tegmental area are implicated in the control of cognitive processes and emotional behaviour. Lesions with 6-hydroxydopamine of the mesocortical DA system at the terminal level induce learning and retention impairments in a delayed alternation task [8,9]. Lesions of the limbic DA terminals within the nucleus accumbens lead to perserverance, impairment of both ongoing behaviour and investigatory exploration and more generally to impairment in the functional processes leading from motivation to action [8].

These two DA systems are also activated during stressful situations. Thus, an increase in DA turnover in mesocortical and mesolimbic neurons has been observed following electric shock [6], environmental stimuli previously paired with inescapable footshock [5], or exposure to a new environment [11].

Behavioural and physiological changes in response to aversive stimulation have been shown to be dependent not only on the amount of physical stress but also on the possibility of actively controlling the stressor. Typically, inescapable shock but not escapable shock results in a performance deficit in subsequent active escape tasks [7], a higher incidence of gastric ulceration [15] and a reduction of brain norepinephrine levels [10,16].

Control over shock may be achieved not only by behaviour directed at the source of stimulation (i.e., escape-avoidance responding) but also by behaviour redirected toward specific objects present in the environment. For example, the administration of brief electric shocks to the feet of pairs of rats induces an upright posture with both animals facing each other ("boxing") and violently striking with the forepaws [12]. This phenomenon, known as shock-induced fighting, results in a decrease in both plasma ACTH levels [3,14] and blood pressure as opposed to increases if the rats are shocked individually [17].

The present investigation was initiated to study the influence of the social setting under which shock occurs on responses of DA brain systems to footshock. Although no difference was found between rats shocked in pairs and rats shocked individually, there was evidence that brain DA activity was sensitive to social influences.

METHOD

Male Sprague-Dawley albino rats obtained from IFFA-CREDO (Lyon) and weighing 300–320 g were used as subjects. They were housed in plastic cages in groups of three, with food and water ad lib. Lighting conditions were 12 hr on/off with the light phase of the cycle beginning at 7.00 hr. Experiments were conducted between 8.00 and 13.00 hr.

Shock was delivered in a 30×30×40 cm opaque plastic chamber with a transparent cover to enable visual observation of animals. The chamber was housed within a well-lit sound attenuated cabinet. A constant current shock generator (Campden Instruments, model 521/CS) was used to deliver scrambled footshocks to the chamber in which two

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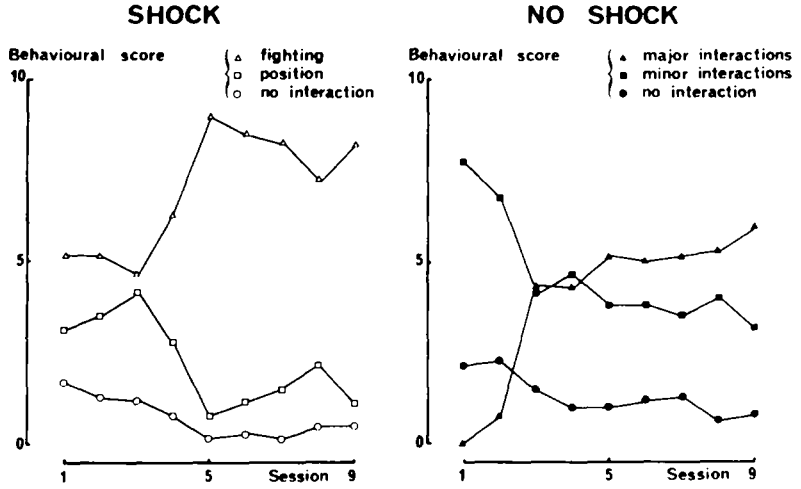

FIG. 1. Evolution of behavioural attitudes in pairs of rats exposed to the test cage with (left) or without electric footshock (right). The vertical axis represents the behavioural score and the horizontal axis the successive daily sessions. Each point is the mean of 6 pairs of rats.

rats were put together. Rats shocked individually were placed in two chambers wired in parallel to another shocker. Every 45 sec, 10 trains of 10 shocks (2mA, 0.5 sec on, 1.5 sec off) were delivered to the stainless-steel grid floor of the chamber. The grid bars were 0.5 cm wide and spaced 2 cm center to center.

The animals were left undisturbed in their home cage for one week after their arrival in the laboratory, after which they were handled and placed individually in the test chamber for 10 min, during 7 daily sessions. At the end of this habituation period, rats were divided randomly into thirteen groups, with 6 animals per group (2 cages). The factors under study included: acute (one session) versus chronic (10 successive sessions) exposure to the test conditions; shock versus no shock (placement into the experimental chamber without any shock); individual versus pair (one or two animals placed into the chamber); before versus after the experimental session, in order to enable comparison of preshock to postshock physiological values. Members of each pair were weight matched so that the difference in weight was less than 10 g. Pairs of rats were made with animals coming from two different home cages.

During the test session, an observer classified the behaviour of each pair of animals into one of the following three mutually exclusive categories. For animals shocked in pairs, the observer rated the behaviour during each train of shock as "no interaction" (mainly escape attempts or freezing postures), "position" (mutual upright posture without physical contact) or "fight" (mutual upright posture accompained by striking with forepaws) [10]. For animals placed in pairs into the chamber without shock, the behaviour was scored every minute as "no interaction", "minor interactions" (mutual sniffing and/or grooming) or "major interactions" (offensive/defensive posture). In each case, the subordinate member of the pair was identified as the rat which displayed a supine posture with the other rat (the dominant) arched over it. The reliability of scoring was regularly checked by a second observer. For each group, data were expressed as number of episodes of the different behavioural categories per session.

Animals were sacrificed by decapitation and their brains quickly removed and cut into 1 or 2 mm thick coronal slices perpendicular to the dorsal surface of the brain. Slices were placed on a refrigerated stage and brain structures corresponding to limbic and cortical dopamine projections were dissected out [5] and put onto dry ice. Brain samples were stored at −70°C until the biochemical analysis. Brain DA and dihydroxyphenylacetic acid (DOPAC) were measured by a radioenzymatic method [5]. Tyrosine hydroxylase (TH) enzymatic activities in the accumbens were determined on the supernants collected after centrifugation of sonicated tissue homogenate in tris acetate buffer. Enzymatic activities were assessed by measuring the rate of formation of 14C-CO2 from 1-(1-14c)tyrosine, according to Waymire *et al.* [13].

Statistical analysis was performed using one- or two-way analysis of variance followed by a Newman-Keuls' test for multiple comparisons.

RESULTS

In both conditions (shock and no shock), paired animals displayed instances of agonistic behaviour (Fig. 1). The incidence of shock-induced fighting (mutual upright posture accompanied by striking with forepaws) and major interactions in non shocked pairs (offensive/defensive posture) increased across sessions, $F(8,40)=4.66$ and 5.19 respectively, $p<0.001$. Position postures in shocked pairs (mutual upright posture without physical contact) and minor interactions in non shocked pairs (mutual sniffing and/or grooming) correlatively decreased, $F(8,40)=2.93$, $p<0.05$ and 4.74, $p<0.001$ respectively. The number of episodes in which no interaction was scored remained stable across sessions $F(8,40)=1.95$ and 1.62. In shocked rats, in 5 pairs out of 6, a clear dominance order was established by the 3rd or 4th session.

Acute exposure to electric shock induced an increase in DOPAC levels in the three brain structures investigated (accumbens: $F(4,24)=5.86$, $p<0.01$; amygdala: $F(4,25)=3.57$, $p<0.05$; frontal cortex: $F(4,24)=7.57$, $p<0.01$) (Fig. 2). Pairing of two animals in the test cage without shock resulted in a near significant ($p<0.10$) increase in DOPAC levels in the accumbens and frontal cortex, in comparison to controls

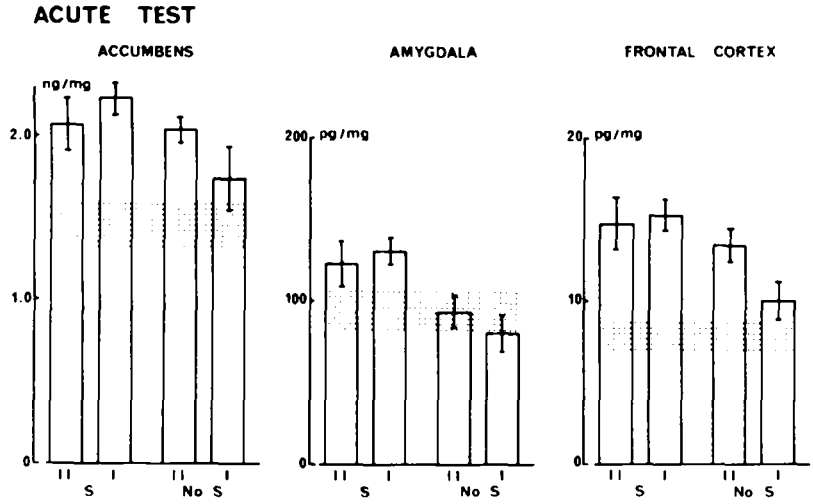

FIG. 2. Mean (±s.e.m., n=6) levels of DOPAC in accumbens, amygdala and anterior frontal cortex of rats exposed either to electric shocks (S) or to the test cage without electric shock (NoS), individually (I) or in pairs (II). The stippled area represents the range of variation (mean±s.e.m., n=6) of DOPAC levels in control rats sacrificed before the experimental session.

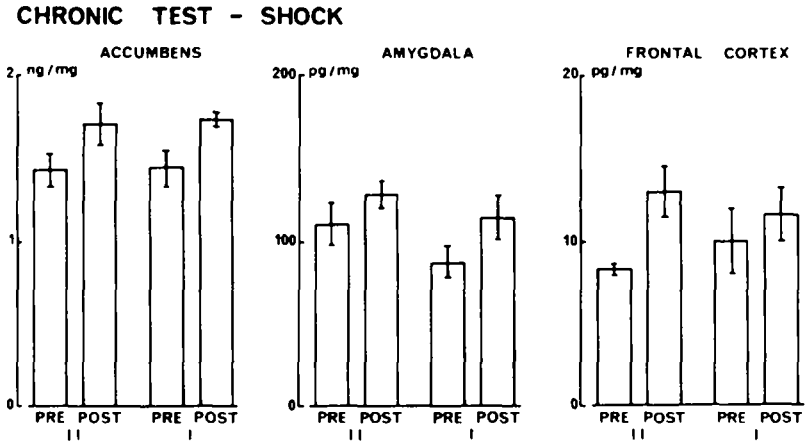

FIG. 3. Mean (±s.e.m., n=6) levels of DOPAC in accumbens, amygdala and anterior frontal cortex or rats exposed chronically to electric shocks either individually (I) or in pairs (II) and sacrificed before (pre) or after (post) the 10th experimental session.

sacrificed before the experimental session. DA levels were not significantly altered in either structure, except in the accumbens where DA levels were lower in animals placed individually in the chamber without shock, in comparison with animals shocked individually ($p<0.05$).

Brain DOPAC (Fig. 3) and DA levels in animals chronically exposed to footshock and killed after the tenth session or just before it were submitted to a 2-way analysis of variance (social environment: pair versus alone; sacrifice time: before versus after the test session). Animals exposed to shock either individually or in pairs exhibited an increase in DOPAC levels in the three brain structures investigated (accumbens: $F(1,20)=6.81$, $p<0.05$; amygdala: $F(1,20)=4.46$, $p<0.05$; frontal cortex: $F(1,20)=4.34$, $p<0.05$). Increases in DOPAC levels in accumbens were attenuated in animals chronically exposed to shock in comparison with animals acutely exposed to shock, $F(1,22)=21.03$, $p<0.01$. The same trend was observed in the frontal cortex but just failed to reach significance, $F(1,22)=3.42$, $p<0.10$. DA levels were not significantly altered after chronic shock exposure in any of the structures investigated.

In animals not exposed to shock but repeatedly put into the test cage either in pairs or individually, the only significant alterations concerned DOPAC (Fig. 4) and DA levels in the accumbens. The interaction social environment × sacrifice time was significant for DA levels, $F(1,20)=5.77$, $p<0.05$, and nearly reached significance for DOPAC levels, $F(1,20)=4.11$, $p<0.10$: both DA and DOPAC levels increased in animals exposed in pairs to the test cage, but did not change in animals exposed alone.

TH activities in accumbens of chronically tested animals were not influenced by shock, $F(1,44)=0.49$, nor by social environment, $F(1,44)=2.41$. However, in the 5 pairs of animals shocked together in which a clear dominance order

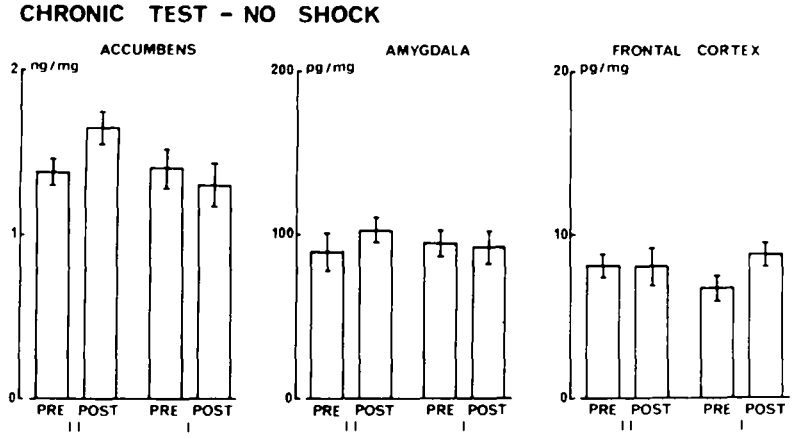

FIG. 4. Mean (+s.e.m., n=6) levels of DOPAC in accumbens, amygdala and anterior frontal cortex of rats exposed to the test cage without shock, either individually (I) or in pairs(II) and sacrificed before (pre) or after (post) the 10th experimental session.

was apparent, dominant subjects had higher TH activities (1.20±0.14 nmoles of product formed per hour and per mg of protein) than subordinates (0.55±0.097 nmoles) and the difference was highly significant, $F(1,4)=35.4$, $p<0.001$. In contrast, in pairs of non shocked animals, TH activities were the same whatever the respective rank of each member of the pair (mean TH value = 1.07±0.16 $F(1,4)=0.15$).

DISCUSSION

This study investigated the influence of the social environment under which footshock occurs on dopamine metabolism in various regions of the rat brain.

Repeated exposure of pairs of rats to electric shock resulted in increasing levels of fighting and the development of a dominance order between the two members of the pair. Rats repeatedly brought together by pairs in a neutral environment also exhibited marked increases in aggression score over days of testing. An increased incidence of attack with repeated testing has already been observed in rodents [2] and is believed to reflect the reinforcing effects of fighting [14].

The increases in DA metabolism after footshock found in the different structures investigated are in general agreement with previous reports [5,6]. In addition, the present study indicates that central DA systems are activated not only by electric shock but also by social stimuli. Such a possibility had already been recognized, based mainly on indirect evidence from studies with dopaminergic agonists and antagonists [4]. The observation that accumbens TH activity decreased in subordinate shocked rats but not in subordinate non shocked rats, despite a high level of aggressive interactions in this last group, cannot be fully explained at present. It provides further evidence for the sensitivity of brain DA systems to social factors.

Most studies on physiological correlates of shock-induced fighting have emphasized the beneficial effects of fighting on the physiological activation provoked by uncontrollable electric shock. Among other things, animals shocked individually suffer a rapid depletion of brain stem norepinephrine, in contrast to rats shocked in pairs [10]. In the present experiment, there was no evidence of a differential activation of dopamine terminals in the brain structures investigated, in animals shocked in pairs as compared to animals shocked individually. Conflicting results exist on the influence of shock controllability on DA levels [1,16]. However, brain DA levels are a poor indicator of DA metabolism, in contrast to DOPAC, which is the main metabolite of brain DA. Changes in DOPAC levels have been observed in response to various physical and psychological stressors, so that the sensitivity of this index is unlikely to be in question. For example, rats put back into cages in which they had been previously shocked displayed a differential activation of DA terminals in the frontal cortex [5]. Since there is evidence that chronic exposure of pairs of rats to inescapable electric shocks leads to differential peripheral physiological activation [3, 14, 15, 17], the present data indicate that fighting in response to shock, unlike social stimuli by themselves, has no direct influence on changes in DA utilization induced by footshock.

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