Pharmacology Biochemistry & Behavior, Vol. 25, pp. 41–44, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Altered Neurobiological Responses to Acute Immobilization in Social-Isolated Mice

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Received 17 December 1985

OEHLER, J., M. JÄHKEL AND J. SCHMIDT. *Altered neurobiological responses to acute immobilization in social-isolated mice.* PHARMACOL BIOCHEM BEHAV 25(1) 41–44, 1986.—Social isolation results in dynamic changes of neurobiological functions. The altered internal state of the CNS is reflected in changes in spontaneous behavior, changed responses to transmission-related substances and changed responsiveness to an additional impairment of normal relations between the organism and its environment. We analysed the influence of an acute 2-hour immobilization on such isolation-dependent changes. Whereas the susceptibility to pentetrazole-induced seizures increased continuously with lengthening of isolation and was not affected by the additional impairment, the responsiveness to transmission-related substances changed dynamically depending upon isolation-induced alterations. An immobilization-induced increase in responsiveness to LSD and propranolol was demonstrable in grouped controls and after short-term isolation. The apomorphine stimulation during prolonged isolation experienced a down- and an upregulation which were repeated in a stronger manner by immobilization responses especially after long-term isolation. It is suggested that the dynamics of isolation-induced changes coincided with changed acute adaptive functions.

Social isolation Acute immobilization Adaptability Climbing Seizure Dopaminergic Noradrenergic Serotoninergic

PROLONGED rearing of rats and mice in isolation leads to altered functional states demonstrable by changed vegetative, hormonal and central nervous system events [1, 3, 7, 8, 15, 20], which subsequently result in altered behavior [8, 10, 19]. In earlier investigations, we have found that isolation-induced changes develop time dependently. We have demonstrated clear dynamic courses for behavioral changes such as enhanced locomotion and aggression [10]. Our pharmacological studies have shown that transmitter-related changes are different after several periods of isolation [9]. In this respect, the question arises regarding what such alterations mean for adaptive and regulative function, for instance in response to acute impairing influences (stress). Several investigations suggest reduced stress responses after social isolation. Nishikawa and Tanaka [6] found in isolated rats a decreased jumping to footshock. This was explained as an enhanced pain threshold [4,12] and was attributed to a decrease in opiate binding sites by Kostowski *et al.* [4] and Schenk *et al.* [14]. Stern *et al.* [15] observed, in isolated rats, less gastric ulceration than in grouped controls, without any change in hormones. In our investigations, we used the social isolation model as long-term impairment with defined behavioral and transmitter-related alterations to study the influence of an acute immobilization impairment. During a six-week isolation period, we analysed the spontaneous climbing behavior, the dopaminergically, serotonergically and noradrenergically affected climbing behavior as well as the pharmacologically induced seizure disposition combined with a 2-hour immobilization. It is shown that the effects of an acute impairment are influenced by several periods of isolation in a different manner.

METHOD

Male albino mice, strain AB Jena (Hirsch Heidenau, GDR) were used when they were 4 weeks old. The animals were subjected to isolation for 1, 7, 21, or 42 days as earlier described [8] or were raised in groups of ten animals in standard cages as controls.

Immobilization was carried out in small copper gauze tubes (2.5 cm in diameter and 12 cm in length) where the animals could not turn, but breathing remained unaffected. Food and water intake was impossible during immobilization. Isolated and control animals were quickly transferred from their home cages to the tubes where they remained for two hours. The climbing experiments began 30 minutes after immobilization.

The spontaneous climbing of mice was measured after a 30-minute habituation period had passed in climbing cages. In agreement with Protais *et al.* [11], we scored the climbing during five-minute periods in the following manner: At the beginning of each minute we determined: 0—mouse with all paws on the floor; 1—with forepaws on the climbing wall; 2—with all paws on the climbing wall.

After 40 mg/kg pentetrazole (Jenapharm) IP, seizures were evaluated using the following scale: 1—tail heaving up

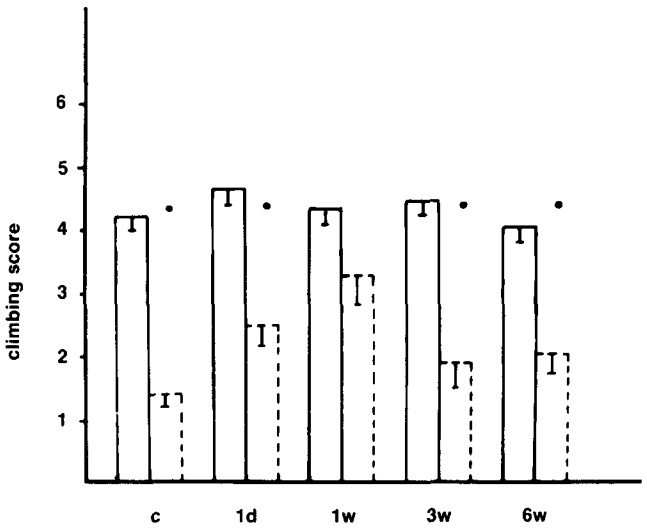

FIG. 1. Changes of climbing behavior by a 2-hour immobilization (---) in group-housed (c) and 1 day (1d), 1 week (1w), 3 weeks (3w) or 6 weeks (6w) isolated mice. Mean±SEM n=100–200 per experiment, ●=$p<0.01$.

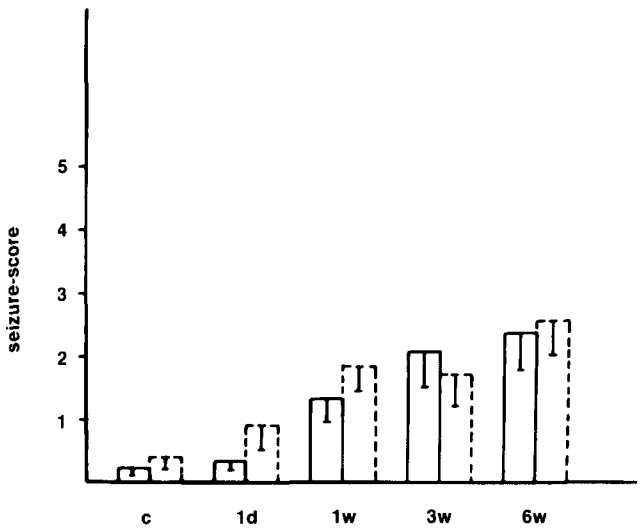

FIG. 2. Changes of seizure-susceptibility by a 2-hour immobilization (---) after 40 mg/kg pentetrazol in group-housed (c) and 1 day (1d), 1 week (1w), 3 weeks (3w) or 6 weeks (6w) isolated mice. Mean±SEM, n=16–18 per experiment.

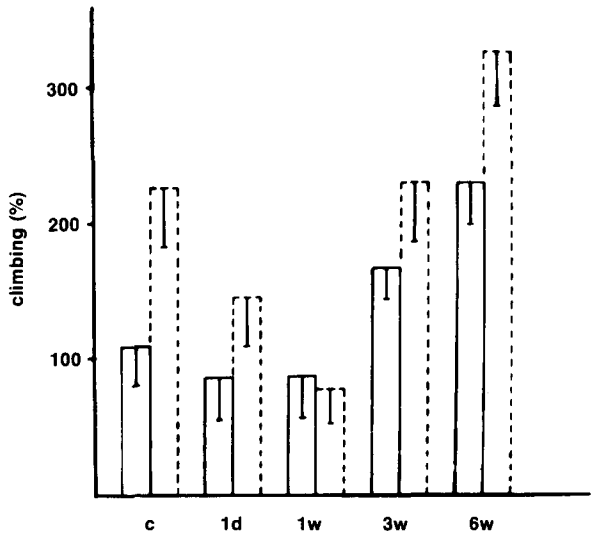

FIG. 3. Changes of climbing behavior by a 2-hour immobilization (---) after 1 mg/kg apomorphine in group-housed (c) and 1 day (1d), 1 week (1w), 3 weeks (3w) or 6 weeks (6w) isolated mice. Mean±SEM, n=16–18 per experiment.

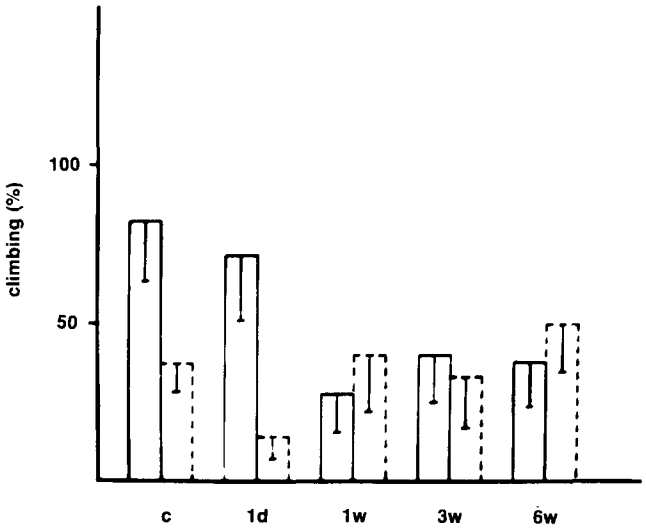

FIG. 4. Changes of climbing behavior by a 2-hour immobilization (---) after 1 mg/kg propranolol in group-housed (c) and 1 day (1d), 1 week (1w), 3 weeks (3w) or 6 weeks (6w) isolated mice. Mean±SEM, n=14–18 per experiment.

or head twitches, 2—struggle with forepaws, 3—set up on hindpaws 4—set up on hindpaws and falling back, 5—generalized seizures.

The transmission-related substances, apomorphine (1 mg/kg), LSD (0.2 mg/kg) and propranolol (1 mg/kg), were tested in climbing experiments and their influences on the climbing behavior were evaluated 10 minutes post-immobilization (p.i.) in the manner above described. In all experiments, the Student's t-test was used for statistical evaluation.

RESULTS

Behavioral Changes

Spontaneous climbing behavior was generally less for immobilized animals. This means that smaller climbing scores were measurable after immobilization in controls as well as in isolated animals. But an increase in climbing for immobilized animals was seen after one day and after one week of isolation compared to the immobilized, grouped controls and to immobilized mice after 3 and 6 weeks of

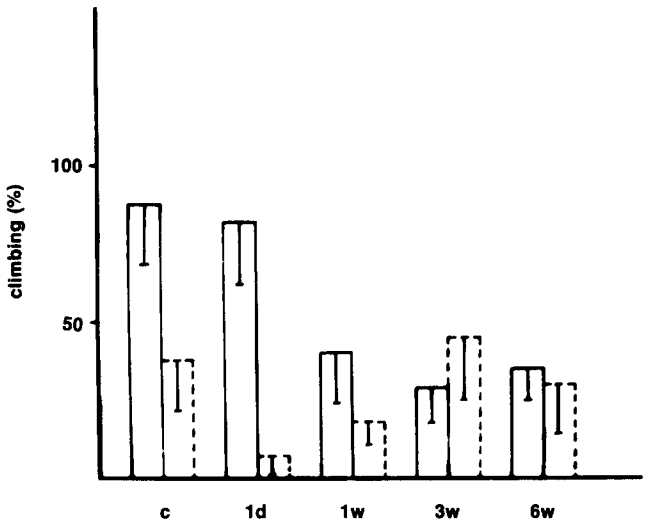

FIG. 5. Changes of climbing behavior by a 2-hour immobilization (---) after 0.2 mg/kg LSD in group-housed (c) and 1 day (1d), 1 week (1w), 3 weeks (3w) or 6 weeks (6w) isolated mice. Mean±SEM, n=15–19 per experiment.

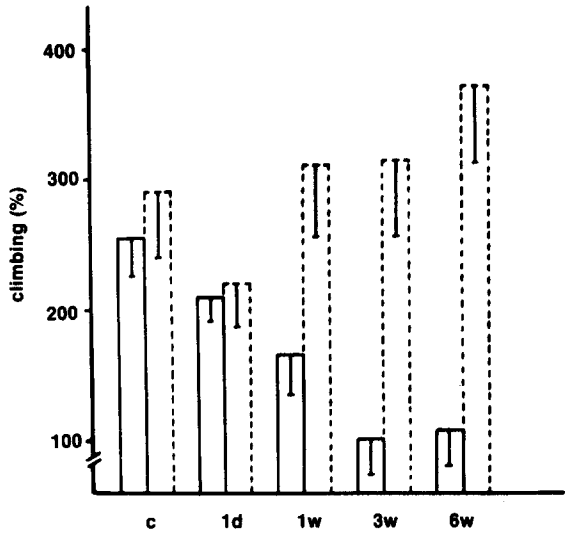

FIG. 6. Changes of climbing behavior by a 2-hour immobilization (---) after 1 mg/kg apomorphine combined with 0.2 mg/kg LSD in group-housed (c) and 1 day (1d), 1 week (1w), 3 weeks (3w) or 6 weeks (6w) isolated mice. Mean±SEM, n=18 per experiment.

isolation. The climbing behavior of group housed and isolated animals showed no time dependence (Fig. 1).

Seizure Changes

Pentetrazole induced seizures showed no immobilization dependent differences in grouped controls. The increase in seizures with increasing duration of isolation was also not influenced by acute immobilization (Fig. 2).

Pharmacological Investigations, Apomorphine

In grouped controls, immobilization led to more climbing induced by a single dose of 1 mg/kg apomorphine. This difference was less after one day and was not to be seen after 1 week of isolation. With lengthening of the isolation, more climbing was found again. It should be noted that the effect of apomorphine to stimulate climbing was also characteristic without immobilization after long-term isolation (Fig. 3).

LSD

In grouped controls, the reduction of climbing induced by a single dose of 0.2 mg/kg LSD was significantly stronger after a 2-hour immobilization. This immobilization effect was most pronounced after one day of isolation. With prolonged isolation, this immobilization influence was not seen (Fig. 4). It should be mentioned that the depressive effect of LSD on climbing increased in any case with prolonged isolation.

Propranolol

After a 2-hour immobilization, a single dose of 1 mg/kg propranolol resulted in greater depression of climbing behavior in grouped controls as well as in mice isolated one day (Fig. 5). After longer isolation periods (1, 3, 6 weeks), no significant differences could be detected in response to propranolol between the immobilized and not immobilized animals. A greater decrease in propranolol-induced climbing by prolonged isolation was noted.

Apomorphine and LSD

The apomorphine stimulation (1 mg/kg) which was potentiated by concomitant administration of LSD (0.2 mg/kg) was not influenced by a 2-hour immobilization in grouped controls and in mice isolated for one day. After prolonged isolation the LSD-potentiation is not seen [7]. However, an additional immobilization always caused a marked apomorphine stimulation by LSD independent on the time of isolation (Fig. 6).

DISCUSSION

Neurobiological alterations induced by social isolation may produce functional changes as a result of the prolonged impairment of the relations between the organism and its environment. It seems that dependent on isolation periods, some of the central nervous subsystems play a different role. During prolonged social isolation the responsiveness to transmission-related substances changed specifically. In earlier investigations, we tried to characterize the behavioral and transmission-specific changes according to their onset and duration during prolonged isolation. There is a difference between immediately beginning short-term changes and persisting alterations starting only after a longer latent period [9]. These investigations refer to the evaluation of such changes and their influence on neurobiological reactivities produced by an acute 2-hour immobilization.

In grouped controls the immobilization caused more intensive responses to apomorphine, propranolol and LSD. In accordance with some stress responses of the CNS [2, 5, 13, 16–18] these responses to immobilization suggested acute regulation properites which, under our conditions, reflect an upregulation of dopaminergic, noradrenergic and serotonergic mechanisms. The immobilization effects observed during different isolation periods suggested an interference between processes caused by acute and long-term impairments. Short-term and immediately starting processes at the beginning of isolation [9] coincided, in some cases, with mech-

anisms similar to stress responses. The facilitated responses to propranolol and LSD can be considered to prove these sensibilisations. After one week, i.e., after the onset of persistent isolation-dependent changes [9], the reactions to transmission-related substances were specifically affected by an additional acute immobilization.

Compared with shorter or longer isolation periods, the immobilization induced depression of spontaneous climbing was less than after only one week of isolation. This can be considered a particularly interesting aspect, because we observed a reduced sensitivity in dopaminergic mechanisms at the same time. However, the relations between the spontaneous behavioral changes and the proven sensitivity alterations are still unclear. Other interferences confirm the idea that acute responses depend on the dynamics of isolation-related long-term functional alterations. After prolonged isolation (3 and 6 weeks), when the depression of climbing was stronger by LSD or propranolol and reflected the isolation-induced altered sensitivities, an additional effect was not seen in response to acute immobilization. However, the isolation-dependent strong dopaminergic stimulation by apomorphine was further enhanced by acute immobilization in 3 and 6 weeks isolated mice. Selective changes of dopaminergic mechanisms, especially in mesolimbic and cortical structures, in response to acute or chronic impairments, have already been reported by Thierry *et al.* [17] and Tissari *et al.* [18]. Our results underline that the activation of dopaminergic mechanisms by acute impairment varied in dependence on the dynamic alterations of dopaminergic mechanisms induced by long-term isolation [9]. Since the functionally relevant structures were characterized not only by single transmission-specific mechanisms, an investigation of transmitter interactions and integrative mechanisms is advisable. In the case of isolation rearing the increase in apomorphine effects by LSD was attenuated [7]. After a subsequent acute immobilization the LSD-induced increase in apomorphine effects was independent on the duration of the foregoing isolation housing. Therefore the two cases reflected different integrative states of the dopaminergic and serotoninergic mechanisms.

During prolonged isolation periods of 6 weeks, complex changes developed also, which can only be explained by a continuously enhancing sensibility. These mechanisms are not influenced by acute immobilization-activated processes. One example is indicated by the continuously enhancing pentetrazole-induced seizures. Similarities can be seen when analysing the isolation-induced aggressive behavior (unpublished). According to our view, the sensitivity changes in several mechanisms during 6 weeks of isolation especially the changes observed after one week, seem to interesting. Within one week only, specific isolation-dependent alterations became manifest. Possibly the changed sensitivities, occurring particularly after one week as a response of isolated animals to acute impairments, reflected an especially "vulnerable" period. According to decreased pain thresholds and pain reactions described by Nisikawa and Tanaka [6], a decrease in the adaptability of isolated animals can be assumed. Possibly after prolonged isolation restrained alterations of some dynamic mechanisms affect acute stress responses specifically.

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