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Social Isolation: Effects on Pain Threshold and Stress-Induced Analgesia

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PUGLISI-ALLEGRA, S. AND A. OLIVERIO. *Social isolation: Effects on pain threshold and stress-induced analgesia.* PHARMACOL BIOCHEM BEHAV **19**(4) 679–681, 1983.—Individually housed DBA/2 mice showed higher pain thresholds than grouped mice. Stress-induced analgesia was evident in grouped but not in isolated mice. Since also morphine injections did not result in analgesic effects in isolated mice, it is suggested that social isolation results in an increased release of opiods which may produce a decreased sensitivity at the opiate receptor level. The role of endogenous opioids in relation to social isolation is discussed.

Social isolation Stress Analgesia Morphine Naloxone Mice

SOCIAL isolation in rodents results in a number of behavioral modifications ranging from increased aggression to altered reactivity to external stimuli [15]. A number of experiments were focused on the neurophysiological and neurochemical mechanisms underlying social isolation. In particular the role of catecholaminergic [15], serotonergic [15,16] and GABAergic systems [14] was studied. More recently possible interactions between endogenous opioids and social isolation have been envisaged since opioids play a role in a number of emotional mechanisms [1,11] and stressful events [1, 4, 10, 12].

Different types of acute stressors produce an increased release of opioids resulting in analgesic effects [1,4]. Unlike acute stress, which reduces pain responsiveness, prolonged exposure to stress leads to enhanced reactivity to pain [1,4]. Such effect was explained in terms of a chronic reduction in the functional availability of endorphin [9]. Exposure to chronic stressors represents an interesting model for depressive and psychotic disturbances since a continuous activation of peptidergic and related dopaminergic synapses may result in functional alteration ranging from exhaustion of the system to receptor hypersensitivity [10].

The role of social isolation in terms of stress has been debated [3,6]. Its effects differ depending on the environmental conditions, species and strain of animals considered [15]. Since strain differences in the reactivity to social isolation have been described [5, 7, 14], in the present research we decided to use socially isolated DBA/2 mice since they are particularly reactive to individual housing [14]. The basal levels of pain thresholds, sensitivity to morphine and the effects of stress-induced analgesia were assessed in grouped or isolated mice in order to investigate the role of endogenous opioids in relation to isolation.

METHOD

Male DBA/2 mice (Charles River, Como, Italy) (n=100) aged 11–12 weeks and weighing 21–23 g at the beginning of the experiments were used. They were either individually housed (isolated) in opaque plastic cages (27×21×13.5 cm) or housed in groups of 6 animals (grouped) per standard breeding cage of the same dimension. For 8 weeks the mice were maintained with food and water ad lib in a 12/12 hr light-dark cycle and tested during the light period. The duration of isolation was decided on the ground of previous experiments [14].

Immobilization stress was produced by placing the animals in a snug-fit Plexiglas restraining apparatus, similar to that described by Amir *et al.* [2]. The mice were immobilized for 60 min. After 45 min of immobilization they were taken out from the apparatus and injected with drugs or saline. Then they were put in an apparatus for a further 15 min immobilization period.

The mice were subjected to a tail-flick latency test immediately after the 60-min immobilization. The nociceptive stimulus was radiant heat. The mice were immobilized during testing by restraining them by hand and given a single trial. All the groups were tested on the same day within a period of 3 hours. Each group consisted of ten animals. Mice were injected with morphine (5 mg/kg) and naloxone hydrochloride (5 mg/kg) (Endo Lab., NY). Drugs were administered intraperitoneally (in a volume of 10 ml/kg) in a 0.9% saline, 15 min before tail-flick tests. The performance of each treated group was compared with that of saline injected (control) groups.

Data were statistically analyzed by three-factor analysis of variance (ANOVA), the factors being differential housing (2 levels=group and isolation), stress condition (2 levels =stress and no stress), and drug treatment (2 levels=saline and morphine). Further analyses for individual between-group comparisons were carried out with post hoc tests by employing the error terms of the overall analysis of variance. Student's *t*-test (two tailed) was also employed. Grouped or isolated DBA/2 mice were used.

TABLE 1

Housing condition	Treatment			
	Controls	Stress	Morphine	Stress + Morphine
Grouped	5.43 ± 0.43	11.24 ± 0.90*	13.22 ± 0.94*	23.78 ± 1.83*†‡
Isolated	13.79 ± 0.94	14.3 ± 0.78	16.38 ± 1.34	14.3 ± 0.54

Tail-flick latencies (in sec) (Mean ± S.E.) of grouped and isolated DBA/2 mice following 60 min immobilization stress and/or injections of morphine (5 mg/kg).

ANOVA showed a significant stress condition main effect, $F(1,72)=24.67$, $p<0.01$, a significant drug treatment main, effect, $F(1,72)=59.18$, $p<0.01$, significant differential housing × stress condition, $F(1,72)=36.25$, $p<0.01$, and differential housing × drug treatment, $F(1,72)=35.45$, $p<0.01$, interactions and a significant differential housing stress condition × drug treatment interaction, $F(1,72)=6.09$, $p<0.05$. Individual between-group comparisons showed significant differences between grouped control stressed mice, mice injected with morphine and stressed mice injected with morphine. Moreover grouped mice stressed and injected with morphine were statistically different from grouped mice injected with morphine and from stressed mice.

No differences among isolated mice were evident.

Significantly different from control (*), stressed (†) and morphine (‡) injected mice ($p<0.01$).

RESULTS AND DISCUSSION

Table 1 indicates that mice that were individually housed (and injected with saline) showed higher pain thresholds than grouped, saline-injected mice ($p<0.01$). A group of isolated mice injected with naloxone (5 mg/kg) showed lower pain thresholds. Their tail-flick latencies were intermediate (8.14±0.85) in comparison to grouped or isolated mice. In fact the performance of isolated mice injected with naloxone was significantly lower than that of saline injected isolated mice ($p<0.01$). Pain thresholds of grouped mice injected with naloxone (5 mg/kg) were not significantly different in comparison with saline-injected grouped mice (saline injected: 5.43±0.43; naloxone injected: 5.10±0.33).

When the effects of immobilization stress are considered, grouped mice showed a sharp decrease of pain sensitivity while a lower, non significant effect was evident in isolated mice: in fact a 140% increase of pain threshold was evident in grouped mice subjected to immobilization stress in relation to non-stressed mice while pain threshold of isolated mice subjected to stress increased of 18% only in relation to isolated saline-injected mice.

When the analgesic effects of morphine are considered a twofold increase in pain threshold was evident in grouped mice and a much higher increase was evident in grouped mice subjected to immobilization stress. On the contrary the same dose of morphine did not result in analgesic reactions in isolated mice. Similarly a lack of analgesic effects was evident in isolated mice subjected to stress and injected with morphine.

In general, the present findings indicate that isolated mice are characterized by a higher nociception threshold. The injection of naloxone resulted in a decrease of pain threshold, thus suggesting that an increased release of endogenous opioids is responsible for the observed increased nociception threshold.

An acute stressor, e.g., immobilization, did not result in increased nociception thresholds in isolated mice while it was evident in grouped animals. An altered release or sensitivity to endogenous opioids may be responsible for the absence of stress-induced analgesia in isolated mice since also the injection of morphine (or morphine + stress) did not increase pain thresholds.

According to Schenk *et al.* [13] long term isolation decreased opiate receptor binding in the brain. This phenomenon may be responsible for the observed decreased sensitivity to morphine in isolated animals [8]. Thus it may be explained by modifications at the receptor level. A cross-tolerance effect between isolation and acute opioid stimulation (as induced by stress) or morphine further supports an involvement of opioid receptors in long term isolation.

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