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γ-Aminobutyric Acid in Brain Areas of Isolated Aggressive or Non-aggressive Inbred Strains of Mice

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SIMLER, S., S. PUGLISI-ALLEGRA AND P. MANDEL. *γ-Aminobutyric acid in brain areas of isolated aggressive or non-aggressive inbred strains of mice.* PHARMAC. BIOCHEM. BEHAV. 16(1) 57–61, 1982.—In order to investigate the effects of social isolation on aggressive behavior and GABA levels in different brain areas, inbred mice of the C57 Bl/6 and the DBA/2 strains were housed individually over a period of 8 weeks. Social isolation induced a clear increase of aggressive responses only in the DBA/2 strain and a decrease of GABA levels in septum, striatum, olfactory bulb and posterior colliculus in both the C57Bl/6 and in the DBA/2 strains. An increase of neurotransmitter concentration was observed in amygdala of DBA mice. DBA mice when compared to C57 mice showed significantly lower levels of GABA in olfactory bulb and striatum. These results are discussed in light of several previous studies which have pointed out a correlation between a deficiency of GABA mediated inhibition in some brain areas and different kinds of aggressive behavior as well as the possibility of a blockade of aggressive behavior by potentiation of GABAergic mediated inhibition. A possible suggestion emerging from our results is that the aggressive responses exhibited by isolated DBA mice but not by isolated C57 mice may be related to lower levels of the inhibitory neurotransmitter in the olfactory bulb and striatum.

GABA Isolation Aggressive behavior Inbred mouse strains

ISOLATION-induced agonistic behavior in mice is used as a tool in the study of molecular mechanisms underlying aggressive behavior.

It has been reported that prolonged isolation produces an increase of brain dopamine turnover, a slight decrease of brain noradrenaline turnover and a large decrease of brain serotonin turnover [19].

In recent years, several studies have pointed out that the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) may play an important role in the control of different kinds of aggressive behavior [7–10]. Concerning isolation-induced agonistic behavior, DeFeudis *et al.* [3] reported that binding capacity of a heavy synaptosomal fraction was lower in the brains of isolated than in those of grouped mice. A decrease of glutamic acid decarboxylase activity in the brains of isolated mice has also been observed [1]. Earley and Leonard [4] reported that the tendency to exhibit aggressive responses is inversely related to GABA concentration in certain brain regions of isolated outbred strains of mice. We considered that inbred strains of mice which present different behavioral patterns and differences in brain chemistry [5, 6, 12] may be a useful tool for the eventual investigation of differences in the GABAergic system and its involvement in the effects produced by different housing conditions.

Recently, it has been reported that after 8 weeks of isolation "aggressive" DBA/2 mice, when compared to "non aggressive" C57Bl/6 mice, are characterized by hyperreactivity, increased motor activity and increased excitability [17].

The purpose of this study was to investigate the effects of individual housing (social isolation) on aggressive behavior and GABA levels in various brain regions of C57Bl/6 (C57) and DBA/2 (DBA) mice.

METHOD

Animals

Male mice (Charles River, France) of the C57 (n=56) and the DBA (n=56) strains, aged 11–12 weeks and weighing 21–24 g at the beginning of the experiment, were used. They were either individually housed (isolated) in opaque cages (27×21×13,5 cm) or 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. The experiments were carried out during the light period.

Aggressive Behavior

Aggressive responses were assessed by means of a

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method previously described [14]. The latency to the first fighting episode, the number and the total time of fighting between two grouped or isolated mice were automatically recorded for a 10 min session. A total of eight pairs of mice for each experimental group were tested.

Brain GABA Levels

Naive grouped and isolated mice of both C57 and DBA strains were used. Mice were killed by focussed microwave irradiation of the head (Litton, LMN, 70/50, 2 KW, 2.45 GHz, 3 sec). Brains were removed, dissected into 15 anatomical areas, and frozen in liquid nitrogen, all as previously described [18]. GABA extraction and determination were performed on the brain areas according to the dansylation method of Seiler and Wiechmann [16]. The stable fluorescent dansyl derivative can be separated from other compounds present by one dimensional chromatography on silicagel G layers/two runs in diethyloxide:cyclohexane, 6:4, v/v) and can be quantitatively determined by direct scanning of the thin layer plates in the range of 0.1 to 5 nanomoles/spot on an Aminco-Bowman spectro-fluorimeter.

Statistics

The results were statistically analyzed by two-factor analysis of variance (ANOVA), the factors being strain (2 levels=C57 and DBA) and differential housing (2 levels =group and isolation). Further analysis for individual between-group comparisons were carried out with post hoc tests by employing the error term of the overall analysis of variance.

Three different two-factor ANOVA for latency to the first fighting episode, the number and the total time of fighting were carried out. A two-factor ANOVA was carried out for each brain area. Each analysis was performed on GABA level values expressed in μmole/g dry weight.

RESULTS

Aggressive Behavior

The effects of social isolation on aggressive responses of C57 and DBA mice are presented in Fig. 1. For the three parameters assessed the two-factor ANOVA showed a significant strain effect, a significant differential housing effect and a significant strain×differential housing interaction. Thus, further between-group comparisons were carried out. The results showed that after eight weeks of individual housing DBA mice exhibited high levels of aggressive responses in comparison with grouped DBA mice. Also isolated C57 mice showed some aggressive responses but they were not statistically different from those of grouped mice. It must be pointed out that number of aggressive responses of isolated DBA mice differed from aggressive responses of isolated C57 mice with statistical significance.

Brain GABA Levels

The overall effects of social isolation on GABA levels in different brain areas of C57 and DBA mice are shown in Table 1. The two-factor ANOVA showed a significant strain effect for olfactory bulb, striatum, hypothalamus, posterior colliculus and frontal cortex. In particular, DBA mice, irrespective of the differential housing showed significantly lower GABA levels in olfactory bulb and striatum and higher GABA levels in hypothalamus, posterior colliculus and fron-

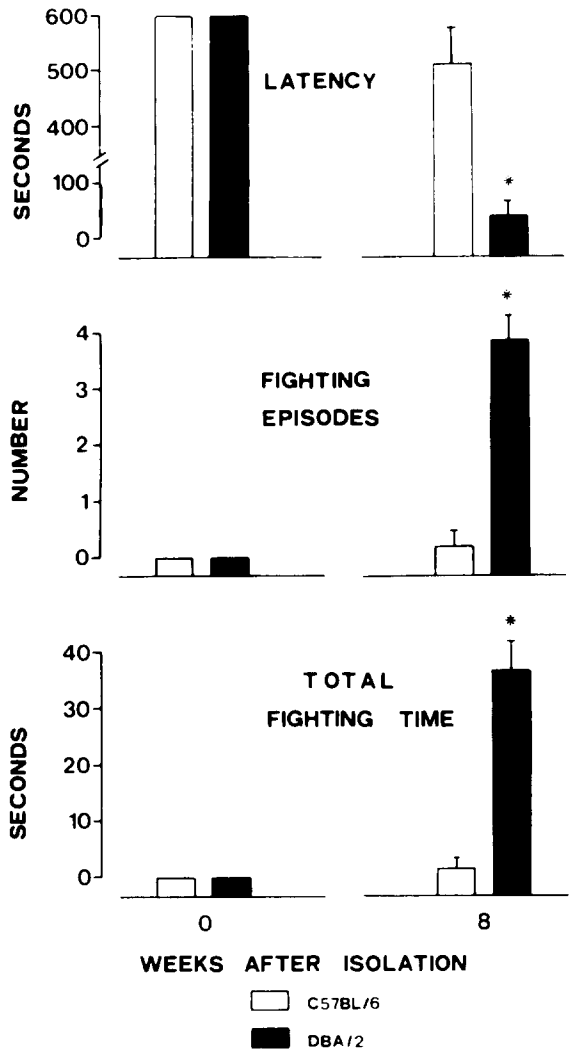

FIG. 1. Effects of social isolation on aggressive behavior of C57 and DBA mice. Aggressive responses were expressed by the latency to the first fighting episode (Latency), the number (Number) and the total time of fighting episodes (Time) during a 10 min. testing session. Data were statistically analyzed by two-factor ANOVA. Those couples of mice that failed to fight during a ten minute experimental session were assigned a maximum latency score of 600 sec. For the three parameters assessed ANOVA showed a significant strain main effect [Latency: $F(1,28)=39.08$, $p<0.001$; Number: $F(1,28)=37.95$, $p<0.001$; Time $F(1,28)=37.34$, $p<0.001$]. A significant differential housing main effect [Latency: $F(1,28)=79.80$, $p<0.001$; Number: $F(1,28)=54.65$, $p<0.001$; Time: $F(1,28)=44.79$, $p<0.001$] and a significant strain×differential housing interaction [Latency: $F(1,28)$: 39.08, $p<0.001$; Number: $F(1,28)=37.95$, $p<0.001$; Time: $F(1,28)=37.34$, $p<0.001$]. Individual between-group comparisons showed significant differences between isolated and grouped DBA mice [Latency: $F(1,28)=115.29$, $p<0.001$; Number: $F(1,28)=91.84$, $p<0.001$; Time: $F(1,28)=81.96$, $p<0.001$] while isolated C57 mice did not differ significantly from grouped C57 mice [Latency: $F(1,28)=3.59$, n.s.; Number: $F(1,28)=0.75$, n.s.; Time $F(1,28)=0.16$, n.s.]. Aggressive responses of isolated DBA mice differed from those of isolated C57 mice with statistical significance [Latency: $F(1,28)=76.16$, $p<0.001$; Number: $F(1,28)=75,90$, $p<0.001$; Time: $F(1,28)=74.68$, $p<0.001$]. *Significantly different from grouped mice of the same strain. $p<0.001$.

TABLE 1

GABA LEVEL IN DIFFERENT BRAIN AREAS IN GROUPED AND ISOLATED C57Bl/6 AND DBA/2 MICE

	C57		DBA	
	Grouped mice	Isolated mice	Grouped mice	Isolated mice
Septum†	13.8 ± 0.6 (7)	11.6 ± 0.6 (7)	14.3 ± 0.8 (7)	11.2 ± 0.5 (7)
Amygdala†	11.1 ± 0.5 (9)	11.1 ± 0.6 (9)	9.5 ± 0.6 (9)‡	12.3 ± 0.6 (9)
Olfactory bulb*†	32.0 ± 1.2 (12)	26.6 ± 0.6 (12)	28.4 ± 0.8 (12)	21.2 ± 2.0 (12)
Cerebellum	4.5 ± 0.2 (6)	5.2 ± 0.3 (6)	4.3 ± 0.2 (6)	4.50 ± 0.3 (6)
Pons	4.9 ± 0.2 (6)	5.2 ± 0.2 (6)	5.3 ± 0.3 (6)	5.2 ± 0.2 (6)
Striatum*†	12.9 ± 0.6 (7)	10.8 ± 0.5 (7)	11.3 ± 1.0 (7)	8.7 ± 0.3 (7)
Hypothalamus*	14.8 ± 0.9 (6)	14.4 ± 0.5 (6)	16.3 ± 0.7 (6)	16.8 ± 1.2 (6)
Thalamus	10.5 ± 0.2 (6)	10.5 ± 0.5 (6)	10.9 ± 0.7 (6)	9.8 ± 0.4 (6)
Anterior colliculus	13.9 ± 0.5 (6)	14.4 ± 0.5 (6)	14.7 ± 0.2 (6)	14.9 ± 1.0 (6)
Posterior colliculus*†	9.5 ± 0.4 (10)	8.5 ± 0.4 (10)	13.8 ± 0.5 (10)	10.9 ± 0.60 (10)
Hippocampus	7.8 ± 0.4 (6)	8.0 ± 0.5 (6)	7.2 ± 0.4 (6)	7.8 ± 0.7 (6)
Substantia nigra	13.4 ± 0.7 (6)	13.0 ± 0.7 (6)	12.1 ± 0.6 (6)	13.5 ± 0.6 (6)
Temporal cortex	6.9 ± 0.3 (7)	6.3 ± 0.5 (7)	6.3 ± 0.1 (7)	6.3 ± 0.2 (7)
Occipital cortex	5.3 ± 0.3 (7)	5.5 ± 0.3 (7)	5.2 ± 0.3 (7)	5.4 ± 0.3 (7)
Frontal cortex*	7.9 ± 0.2 (9)	7.2 ± 0.3 (9)	10.5 ± 0.7 (9)	11.3 ± 0.7 (9)

Results are expressed as μmol/g dry weight ± S.D. Number of animals is expressed between brackets.

A two-factor ANOVA carried out for each brain area showed a significant strain effect for olfactory bulb [$F(1,44)=12.74$, $p<0.001$], striatum [$F(1,24)=7.64$, $p<0.01$], hypothalamus [$F(1,20)=5.08$, $p<0.05$], posterior colliculus [$F(1,36)=51.22$, $p<0.001$] and frontal cortex [$F(1,32)=41.83$, $p<0.001$] and a significant differential housing effect for septum [$F(1,24)=16.57$, $p<0.001$], amygdala [$F(1,32)=5.47$, $p<0.05$], olfactory bulb [$F(1,44)=24.21$, $p<0.001$], striatum [$F(1,24)=12.75$, $p<0.001$] and posterior colliculus [$F(1,36)=16.60$, $p<0.001$]. A strain × differential housing interaction was shown for amygdala [$F(1,32)=5.25$, $p<0.05$]. Individual between-group comparisons showed that in this brain area GABA levels of isolated C57 mice were not different from those of grouped C57 mice [$F(1,32)=0.00$, n.s.]. Isolated DBA mice exhibited significantly higher GABA levels in comparison with grouped DBA mice [$F(1,32)=10.72$, $p<0.01$]. No significant differences were observed between isolated C57 and DBA mice [$F(1,32)=1.97$, n.s.].

*=significant strain main effect.

†=significant differential housing main effect.

‡=significantly different from isolated mice of the same strain ($p<0.01$).

tal cortex in comparison with C57 mice. A significant differential housing effect for septum, amygdala, olfactory bulb, striatum and posterior colliculus was also shown. With the exception of amygdala, individual housing resulted in a decrease of GABA levels in septum, olfactory bulb, striatum and posterior colliculus of both C57 and DBA mice. Moreover, a strain×differential housing interaction for amygdala made necessary further analysis. Individual between-group comparisons showed that in this brain area GABA levels of isolated C57 mice did not differ from those of grouped C57 mice. On the contrary isolated DBA mice showed higher GABA levels in comparison with grouped DBA mice. It must be pointed out that no difference in amygdala GABA levels was shown between isolated C57 and DBA mice (see Table 1).

DISCUSSION

The present results show that in the DBA but not in the C57 strain individual housing resulted in a clear increase of aggressive responses, thus confirming previous results [17].

After isolation both C57 and DBA mice showed a decrease of GABA levels in septum, olfactory bulb, striatum and posterior colliculus. Furthermore, only DBA mice showed an increase of GABA levels in amygdala. It must be also pointed out that irrespective of differential housing DBA mice showed lower levels of GABA in olfactory bulb and striatum and higher levels in posterior colliculus.

Our results are in part consistent with those of Earley and Leonard [4] who found lower levels of GABA in striatum, hippocampus and amygdala of isolated outbred albino mice in comparison with grouped mice and lower levels of GABA in olfactory bulb and striatum of isolated high aggressive mice in comparison with mice exhibiting low levels of aggressive responses. In addition to the aforementioned results concerning septum and amygdala we did not find any difference of GABA levels in hippocampus after isolation in both C57 and DBA mice. These discrepancies of our results with those of Earley and Leonard [4] may depend on strain differences and/or on different experimental procedures. The differences observed within each strain between isolated and grouped mice, as well as those between isolated mice of both strains might be related to the behavioral and physiological differences exhibited by DBA but not by C57 mice after 8 weeks of social isolation [16]: hyper-reactivity, increased motor activity and increased excitability.

Some working hypotheses emerge from the present findings concerning the role of GABA system in the control of isolation induced agonistic behavior. We observed after isolation a decrease of GABA in olfactory bulb, septum, striatum and posterior colliculus in both C57 and DBA mice. Some studies have recently pointed out an inverse relation-

ship between GABA levels in the brain and aggressive responses in different kinds of aggressive behavior. A decrease of GABA level in the olfactory bulb was also observed in muricidal rats [7, 9, 10]. Compensation for this decrease in GABA content of the olfactory bulb produced by local injections of GABA or sodium n-dipropylacetate (DPA), an inhibitor of GABA-transaminase, suppressed killing behavior [9]. A similar inhibition of muricidal behavior was observed after local injection in the olfactory bulb of diaminobutyric acid, an agent that blocks GABA reuptake, or of the GABA agonist, muscimol [10,11]. On the other hand, injections of picrotoxin (a GABA antagonist) or allylglycine (an inhibitor of glutamate decarboxylase) induced killing behavior [10]. Moreover, the decrease of GABA in the olfactory bulb and in the striatum in isolated high aggressive mice in comparison with isolated low aggressive mice was reported by Earley and Leonard [4] as previously mentioned. DaVanzo and Sydow [2] reported that amino-oxyacetic acid and gamma-acetylenic GABA produce a suppression of isolation-induced agonistic behavior in mice and a concomitatnt increase of brain GABA levels. It has also been shown that sodium n-dipropylacetate (DPA), muscimol hydrobromide, nipecotic acid amide inhibit isolation induced agonistic behavior in DBA/2 mice [13]. In another kind of aggressive behavior, i.e., that induced by electric shock in mice, we have recently reported [15] that GABA antagonist, picrotoxin and GAD inhibitor, D-L, allylglycine induced aggressive responses in non aggressive mice while DPA and muscimol inhibit aggressive responses in aggressive mice.

In light of the findings discussed above, our present results indicate that the decrease of GABA levels observed in isolated mice in comparison with grouped mice might be related to the expression of aggressive responses. In particular, isolation by itself produced a decrease in septum, olfactory bulb, striatum and posterior colliculus in both strains of mice studied. However, DBA grouped mice when compared to C57 grouped mice showed significantly lower levels of GABA in olfactory bulb, and striatum as indicated by the significant main strain effect (see Table 1); in addition much lower values were observed in the olfactory bulbs and striatum of isolated DBA mice which manifest aggressive behavior.

Thus, it seems likely that higher aggressive responses exhibited by isolated DBA mice in comparison with isolated C57 mice, in our experimental conditions, are related to the lower levels of the inhibitory neurotransmitter in the olfactory bulbs and striatum of DBA mice. These data are in agreement with those concerning GABA in the olfactory bulbs in muricidal behavior. However it should be kept in mind that GABA is not the only neurotransmitter altered in aggressive rodents. Alterations of serotonin metabolism were observed as well. Nevertheless, in view of former data showing that potentialisation of GABAergic neurotransmission also correct the alteration of serotonin and dopamine in muricidal rats, it is likely that GABA, which is the most widespread inhibitory transmitter, plays a major role in the control of aggressive behavior.

It must be taken into account that the changes of GABA levels in amygdala of DBA mice after prolonged individual housing may be related to some changes of other neurotransmitters in different brain structures and to some regulatory mechanisms which might be related to other neurotransmitters.

Further investigations are necessary in order to clarify the interactions between GABA and other neurotransmitters in various brain regions and the role that these neurotransmitters play in the control of the physiological and behavioral effects of social isolation.

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