

# A Critical Period for Social Isolation in the Rat

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Rats housed in social isolation show heightened levels of object-contact in an open-field and are slower than socially-housed controls to emerge from a small enclosure into an unfamiliar environment. Isolation between 25 and 45 days of age produced an irreversible effect upon object contact but had no lasting effect if between 16 and 25 days or after 45 days. In contrast to object contact, emergence was affected by isolation at any age and the effect was reversed by subsequent social housing. Thus the effects of isolation upon object contact and upon emergence apparently do not depend upon a single underlying variable.

Previous experiments have shown that the behavior of individually-housed laboratory rats in a novel situation differs markedly from that of socially-housed rats. First, isolates are relatively slow to emerge into a novel environment from a small adjacent enclosure (Lore & Levowitz, 1966; Morgan, 1973). Second, if isolates are placed directly into an open-field they show a slower rate of decline of ambulation than do the socially-housed animals (Einon, Morgan, & Sahakian, 1975). Also, if the open-field contains a variety of objects, the isolates show a slower decline in number of object contacts over time (Einon & Morgan, 1976).

Obvious questions that arise are whether these social-isolate differences depend upon the prevailing housing conditions at the time of testing, upon the length of exposure to these conditions, or upon housing conditions at a critical developmental stage. We have investigated these questions by isolating rats at various ages and for various lengths of time. In the first experiment the effect of isolation between 16 and 25 days was compared to that of isolation between 25 and 45 days. In the second experiment, isolation between 25 and 45 days was compared with isolation between 45 and 90 days. In the third experiment, isolation between 25 and 90 days was compared with isolation between 90 and 180 days. In the final experiment controls were provided for certain non-age-related variables. In all experiments the rats were tested for both object contact and emergence at the end of the later of the 2 possible isolation periods.

Several methodological problems may be noted at the outset. Because we could not ensure exact equating of living conditions between rats of very different ages,

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we must recognize that age of isolation is inevitably confounded with differences in the precise nature of the isolation. The possible contribution of the various confounding factors will be assessed at the relevant places in this report.

## General Methods

### Testing Apparatus

For the emergence test the animals were placed inside a 25-cm length of 9-cm diameter opaque brown plastic ("Bakelite") tubing. The tube was placed in one corner of a 50 x 30 x 11 cm metal box with an open top. The open-field used for the object-contact tests measured 75 x 75 cm and was constructed of Bakelite with a mid-grey oil cloth covering on the walls (40 cm high). Underneath the floor, which consisted of .6 cm steel rods placed .6 cm apart, was a grid of orange-colored wire marking the field out into 25 equal squares (15 cm each side.) The field was lit by a 40-W bulb placed 50 cm above and to one side of the field. The following objects were spaced evenly around the floor: a plastic flower pot (10-cm diameter at the open end); an open-ended cardboard tube (9-cm diameter, 12 cm long); and open-ended Bakelite tube (9-cm diameter, 25 cm long); the lid of a mouse cage (North Kent Plastics; Model M2); a cylindrical galvanized mesh food hopper (11-cm diameter, 10 cm high); an earthenware dish (6-cm diameter, 4 cm high); 2 pieces of crumpled paper; an aluminum whistle from a kettle; a rubber ball (10-cm diameter) with a handle attached; a plastic ring (5-cm diameter).

### Testing Procedure

In the emergence test the animals were placed singly into the tube which was then introduced into the testing chamber, and the time taken to emerge (all 4 feet) was recorded in seconds with a stop watch. If the rat did not emerge in 3 min the trial was terminated and a score of 180 sec was taken for that animal. In the object-contact test the animal was placed in the center of the field (next to the food hopper) and remained alone in the field for 33 min. Object contact was sampled in 4 separate periods: 0-3 min, 4-7 min, 17-20 min, and 30-33 min. The 4-7 min sample was not taken in Experiment 1. The method of recording object contacts has been described elsewhere (Einon & Morgan, 1976).

The emergence test took place when the animals reached the requisite age (which differed between the experiments), and the object contact test started on the day after.

## Experiment 1

The aim was to detect any lasting effects of isolation between 16 and 25 days in a test carried out at 45 days. Because the time between 16 and 25 days corresponds to a phase of very rapid brain and behavioral development, we might expect this period to

be crucial. Accordingly, half the rats were reared in isolation and half in small social groups from 16 through 25 days. At 25 days half of the isolates were switched to social conditions and the social animals to isolation conditions. The 4 sub-groups were compared for emergence and object contact at 45 days of age.

## Methods

*Subjects and rearing.* Pregnant female hooded rats (Lister, *Rattus norvegicus*) were received from a breeding laboratory (Animal Suppliers, Ltd.) after a 1-hr train journey in stiff cardboard traveling cages. On arrival they were placed in individual plastic breeding cages (45 x 28 x 22 cm) with a supply of paper toweling and wood shavings. Litters were born 4-7 days after arrival. At 16 days *post partum* the males and females within each litter were divided equally into social and isolated conditions. In the case of an odd number of animals the extra animal was placed alternately into the 2 conditions. Despite the considerable preponderance of females in this experiment (32 females, 8 males) equal ratios of males and females were established for the experimental sub-groups.

At 16 days the isolates in each litter were placed individually into small sub-compartments of a mat-black metal box (45.75 x 30.5 cm). Each compartment (15.25 x 15.25 cm) contained cotton-wool bedding, pellets of laboratory chow, a dish of high-protein baby food (Farleys, Ltd.) and the spout of an avian drinking tube, containing milk. The social animals in each litter were placed together (regardless of gender) into one of these compartments. Because of the variable litter size the number of rats comprising each social group varied between 3 and 5. At 25 days of age half of each litter of isolates was re-grouped and half remained in isolation; similarly, half of each group of social animals was switched to the isolation condition and half remained in groups. Reconstituted groups varied between 3 and 5. The social animals were placed in plastic cages (45 x 28 x 22 cm) and the isolates in smaller (33 x 15 x 13 cm) plastic cages. All animals were tested at 45 days according to the procedures described under General methods (see, also, Einon *et al.* [1975].) Each of the experimental sub-groups consisted of 2 males and 8 females giving a total of 40 animals. The sub-groups were designated as follows I-I (isolated throughout), G-G (socially-grouped throughout), G-I (socially-grouped up to 25 days and then isolated), I-G (isolated up to 25 days and then socially grouped.)

## Results

Separate analyses of variance, with conditions before and after 25 days as the 2 main factors, were carried out on the emergence and object-contact scores. In the case of object contact scores repeated measures were analysed over the 3 time samples. Because of the very unequal numbers of males and females no attempt was made to analyze gender differences, but gender was balanced within each sub-group.

*Emergence.* The animals living in isolation at the time of testing (I-I and G-I) took longer to emerge than socially-housed sub-groups ( $F = 10.92$ ;  $df = 1/36$ ;  $p < .01$ ) although earlier isolation, between 16-25 days, was without effect ( $F = 1.43$ ;  $df = 1/36$ ;  $p < .05$ ). A suggestion of interaction between the first and second set of housing conditions did not reach significance ( $F = 3.12$ ;  $df = 1/36$ ). (See Fig. 1.)

*Object Contact.* The first isolation period had no significant effect ( $F < 1.0$ ) but

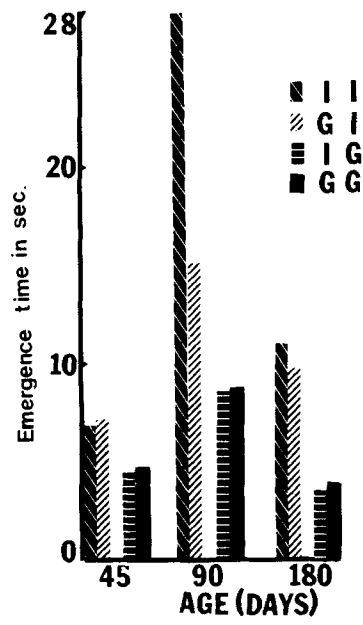


Fig. 1. Time taken to emerge into a novel open-field by isolated (I) and socially-grouped (G) rats tested at 3 different ages (45, 90 and 180 days). The I-I animals had been in isolation since weaning; the G-I animals were initially socially-grouped and subsequently kept in isolation until the test; the I-G animals were initially isolated and subsequently socially-grouped; and the G-G animals were socially-grouped throughout.

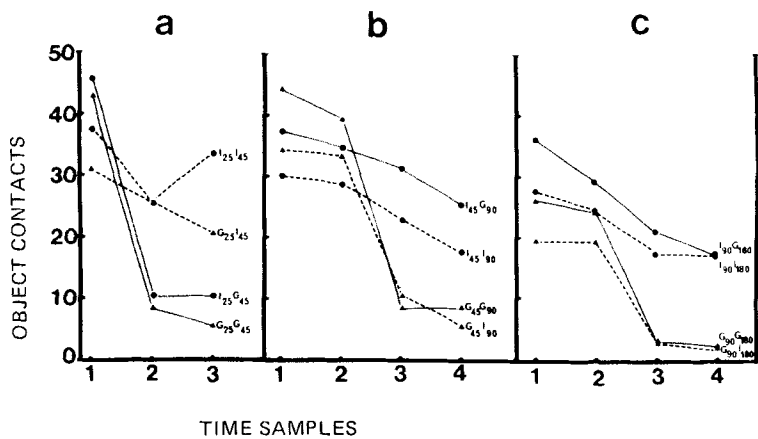


Fig. 2. Object contacts in the open-field made by isolated (I) and socially-grouped (G) rats tested at 3 different ages (45, 90 and 180 days). The animals labelled G<sub>25</sub>I<sub>45</sub> were socially grouped up to 25 days and then isolated between 25 and 45 days. Labels for other animals are to be similarly interpreted.

the second isolation period significantly increased total object contacts ( $F = 5.87$ ;  $df = 1/36$ ;  $p < .05$ .) Moreover, the later isolates (G-I and I-I) showed a lower rate of decline in object contacts over time than their social controls, as indicated by the significant interaction between time samples and the 25-45 day rearing condition ( $F = 22.13$ ;  $df = 2/72$ ;  $p < .01$ .) The interaction between time samples and the first rearing period was not significant ( $F < 1.0$ .) (See Fig. 2.)

## Discussion

The results show that isolation rearing between 16 and 25 days, a crucial period for rat brain development, is neither a necessary nor sufficient condition for lasting effects upon object contact and emergence. We cannot conclude that the 16-25 day period is without effect, for its influence may have been reversed by subsequent social housing. Nor can we conclude that the effects of 25-45 day isolation are permanent, because they too might be reversible by subsequent social grouping. The next experiment was designed to investigate the latter possibility.

## Experiment 2

The 4 groups in this experiment were: I-I (isolated from 25 through 90 days), G-G (socially housed from 25 through 90 days), I-G (isolated from 25 through 45 days and then socially housed up to 90 days), and G-I (socially-housed from 25 through 45 days and then isolated.) All sub-groups were tested for emergence and object contact at 90 days of age.

## Methods

*Subjects and Rearing.* The subjects (24 males and 24 females) came from the same source as in Experiment 1. They were weaned and allocated to conditions at 25 days of age, with each litter being divided equally by gender into the 2 conditions as far as possible. Isolates were placed individually into small plastic cages (33 x 15 x 13 cm) and social animals in groups of 3 into larger plastic cages (45 x 28 x 22 cm.) The social groups were randomly mixed with regard to litter and gender. At 45 days all animals were transferred to wire cages (25 x 29.5 x 19 cm) in the main animal colony. Half the previous social group was placed in isolation. The remaining social animals were reassorted into groups of 3. Each of the new social groups was of a single gender. At 90 days of age object contact and emergence testing was started, using the same procedures as in Experiment 1.

## Results

*Emergence.* The sub-groups housed in isolation at the time of testing (G-I and I-I) were slower to emerge than socially-housed sub-groups ( $F = 12.65$ ;  $df = 1/40$ ;  $p < .01$ .) The effect of the earlier isolation period was not significant ( $F = 3.02$ ;  $df = 1/40$ ; see Fig. 1). Although the main effect of gender was not significant, the interaction between gender and 25-45 days isolation was ( $F = 5.67$ ;  $df = 1/40$ ;  $p < .05$ ). In females this

earlier period of isolation led to slower emergence, whereas in males it had a slight, opposite effect. The effect of the 45-90 period was also smaller in males, but the interaction of gender and later housing was not significant ( $F = 3.18$ ;  $df = 1/40$ .)

*Object Contact.* The earlier isolation period (25-45 days) significantly increased object contact ( $F = 7.48$ ;  $df = 1/40$ ;  $p < .01$ ), whereas the later period (45-90 days) was without effect ( $F < 1.0$ ). The interaction between early housing and time samples was significant ( $F = 27.54$ ;  $df = 3/123$ ;  $p < .01$ ) indicating that isolates showed a slower decline in object contact over time. (See Fig. 2.) The interaction between late housing and time samples was not significant ( $F < 1.0$ ). The only significant effect of gender was its third-order interaction with the early and late housing conditions ( $F = 8.89$ ;  $df = 1/40$ ;  $p < .01$ ).

### Discussion

In Experiment 1 rats isolated between 25 and 45 days were slow to emerge. Because this was not true of the 25-45 days isolates in the present experiment if they had been subsequently housed socially, the effect of isolation apparently was reversed. The effects on object contact were not so labile. The results show that the effects of isolation rearing in the period 25-45 days can last through a subsequent period of social housing. Also, the second isolation period itself was without significant effect on object contact. Taken together, these findings suggest that isolation rearing in the period 25-45 days has more important effects on object contact than later isolation. Some caution is needed here, however, because the conditions of isolation and social grouping differed between the 2 ages. Isolates in the 25-45 day period lived in small plastic cages whereas in the later period they lived in larger mesh cages. If the primary cause of "isolation" effects is perceptual impoverishment, then the earlier period would obviously be the more effective. However, effects of isolation on activity were found in an earlier study (Morgan, 1973) in which isolates were reared in cages the same size as those of the social animals, separated from them by Plexiglas partitions perforated to allow passage of sounds and odors. The fact that isolation before 45 days in those conditions had pronounced effects on activity, whereas in the present experiment isolation between 45 and 90 days did not affect object contact, suggests that age is an important factor. This conclusion would be stronger if in a separate experiment 25-45 day isolation in wire cages is shown to affect object contact (see Experiment 4, below). The problems concerning different cage types do not affect the conclusion that some of the effects of isolation are not reversible. On the contrary, the conclusion is strengthened, for the putatively more enriching wire cages were unable to reverse isolation housing effects if the animals experienced them after 45 days of age. A further implication of the findings is that the effects of isolation are not unitary. Object contact was affected more by earlier than by late isolation, whereas the reverse was true of emergence.

### Experiment 3

The design was identical to that of the previous experiment except that the switch in conditions occurred at 90 days rather than at 45 days and the animals

were tested at 180 days. Our aim was to see if the effects of early isolation could be reversed by a longer period of subsequent social housing.

## Methods

*Subjects and Rearing.* The rats (24 males and 24 females) came from the same source as in Experiment 1. Rearing conditions were identical to Experiment 2 up to 45 days of age. At this age, all rats were transferred to wire cages, but remained in their respective conditions of isolation or social grouping. The social groups were reconstituted so that all members were of the same gender. At 90 days half the isolates were placed in groups of like gender ( $n = 3$ ) and half of each existing social group was isolated. The remaining social animals were reconstituted into groups of 3. Testing on emergence and object contact began at 180 days.

## Results

*Emergence.* Late isolation (90-180 days) increased emergence time ( $F = 15.57$ ;  $df = 1/40$ ;  $p < .01$ ), but the earlier period (25-90 days) was without significant effect ( $F < 1.0$ ). The effects related to gender were insignificant and the complex interaction between gender and early isolation found in Experiment 2 was not repeated. (See Fig. 1.)

*Object Contact.* Early isolation increased object contact ( $F = 31.33$ ;  $df = 1/40$ ;  $p < .01$ ) and caused a decreased decline in contacts over time samples ( $F = 3.99$ ;  $df = 3/123$ ;  $p < .01$ ). The later isolation period (90-180 days), on the other hand, had an effect approaching significance in the reverse direction: the isolates tended to contact *fewer* objects ( $F = 3.04$ ;  $df = 1/40$ ;  $p > .01$ ). The effect was most obvious in the first time sample. (See Fig. 2.) Effects relating to gender were non-significant.

## Discussion

Ninety days of social housing in wire cages failed to obliterate the effects of 25-90 day isolation. (Note that the second half of the isolation 25-90 day period was spent in wire cages, so that I-I and I-G groups spent only 20 days in plastic cages and 135 days in wire cages. If isolation in the plastic cages is critical, the survival of the effects is more remarkable still.) As in Experiment 2, the results show a double dissociation between the effects of early and late isolation, and the tests of emergence and object contact. This reinforces the point that the effects of isolation are not unitary.

## Experiment 4

The final experiment was a control for certain confounding features in the earlier experiments. First, in previous experiments, pre-45 day isolates lived in small plastic cages and post-45 day isolates lived in larger mesh cages. In the present experiment pre-45 day isolates were housed in the latter kind of cage. Second, all social groups in the previous experiments were of mixed gender up to 45 days and of like gender

thereafter. In the present experiment the groups were of like gender before 45 days (only females were used). Finally, all animals in previous experiments were offspring of mothers which arrived in the laboratory while pregnant. The rats in the present experiment were obtained from mothers impregnated in the laboratory.

## Methods

*Subjects and Rearing.* The female offspring ( $n = 20$ ) of Lister hooded rats (Animal Suppliers Ltd.) were weaned at 25 days and placed in wire-mesh cages in the laboratory colony (see Experiment 2 for description of the cages). Isolates were housed individually; social animals lived in cages of the same size as the isolates' in 2 groups of 5. All animals were tested for emergence and object contact starting on Day 45, using the same procedure as in previous experiments.

## Results

*Emergence.* As in previous experiments the isolates were slower to emerge ( $F = 8.01$ ;  $df = 1/18$ ;  $p < .01$ ). Isolates emerged in a mean of 7.5 sec, and the social animals in a mean of 4.9 sec. These times are comparable to those of the I-I and G-G groups in Experiment 1. (See Fig. 1.)

*Object Contact.* As in previous experiments the interaction between housing condition and time samples was significant ( $F = 5.00$ ;  $df = 3/54$ ;  $p < .01$ ), arising because isolates showed a slower decline in object contact over time. The main effect of housing did not reach significance ( $F = 2.75$ ;  $df = 1/18$ ).

## Discussion

The effects of isolation in the present experiment were similar to those of 25-45 day isolation in Experiment 1. In both experiments the housing  $\times$  time samples interaction was more marked than the main effect of housing, indicating that differences in rate of decline of object contact are more important than differences in absolute level. The smaller  $F$ -ratios in the present experiment may be attributed to the smaller sample sizes ( $n = 20$ , versus  $n = 40$  in Experiment 1). Thus isolation in wire cages in the period 25-45 days has similar effects to isolation in small plastic cages. In contrast, isolation in wire cages in the period 45-90 days has little effect upon object contact (Experiment 2). We conclude, therefore, that age of isolation, rather than the type of cage used, was the more important variable in Experiments 1, 2, and 3.

## General Discussion

The results show that isolation has 2 entirely different effects upon the behavior of rats in a novel situation. The first effect, characterized by a slow decline in object contact in the open field, results from isolation before 45 days of age and is not reversed by subsequent social housing. The second effect, a slowness to emerge into a novel environment, is produced by isolation at any age within the range studied,



and is reversible. From the results of Experiment 1, a period of 20 days is sufficient to produce the effect but we have not established how long a period is necessary.

The problem of why isolation after 45 days of age has little effect on object contact can be approached either by considering what maturational changes in the nervous system are complete by this age, or by examining the ontogeny of behavior. Concerning effects of isolation upon brain development, a number of possibilities seem to be ruled out by the finding (Experiment 1) of no permanent effects of 16-25 day isolation. Between 13-25 days the hippocampus becomes functional, the cholinergic and serotonergic systems mature, and by 24 days dopamine levels have almost reached adult proportions (Campbell & Spear, 1972; Jacobson, 1970). Thus the effects of isolation are probably not mediated through these systems. On the other hand, the period of most rapid deposition of myelin (30-40 days; Jacobson, 1963) corresponds quite well to the period in which isolation has seemingly irreversible effects. Thus, isolation housing before 45 days may affect myelination of the rat brain, but this hypothesis has not yet received direct test.

What of behavioral correlates of the "critical period" for isolation? Baenninger (1967) has observed that rats begin to play at about 16 days, that play increases over the next 20 days, and then declines rapidly. In the present experiments we noticed that grouping the isolates at 25 days of age resulted in intense and prolonged rough and tumble play, whereas grouping at 45 or 90 days produced only sniffing and paw contact. Ethologists have supposed that play, such a pronounced feature of the behavior of many young animals, has an important functional significance (e.g. Welker, 1961; Eibl-Eibesfeldt, 1967) and by implication, have suggested that deprivation of play has important consequences. The present findings provide indirect support for this functional interpretation of play, but do not indicate any mechanism whereby play deprivation has such a pronounced effect upon open-field behavior.

If play-deprivation is the important result of social isolation, we might expect that the quality of social interaction, and not merely its quantity, would be the critical variable. We are currently investigating this hypothesis by permitting isolates to play for 1 hr a day with partners whose social responsiveness is manipulated by prior injections of drugs such as amphetamine or chlorpromazine. Our results to date support the play hypothesis, in as much as the effectiveness of the 1 hr a day of contact upon subsequent open field behaviour in the experimental animals depends upon the social responsiveness of the drugged partners (C. C. Kibbler, D. F. Einon, & M. J. Morgan, unpublished data).

## Notes

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