

# Effects of a brief social contact upon open field behaviour and FR 1 escape / avoidance acquisition in socially isolated adult rats

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## Introduction

The idea that social isolation before and after weaning later produced permanent behavioural, psychological and neurological deficiencies in dogs, rats and etc has been experimentally confirmed again and again (Holson & Sackett, 1984 for a detailed review). These reports led the consensus that the experiments using animals as subjects required to pay much attention to the rearing conditions of their early life. Because the interest was mostly confined to the effects of early experience, isolation syndrome in life span seemed to be ignored. As Claassen (1994) pointed out, recognizing the effects of rearing conditions was very important in researching learning mechanisms or endocrine systems because a 1–2 week isolation was the usual procedure as a pre-experimental treatment.

Several researchers have reported that social isolation after maturity would had a little different effects on rats' behaviour, anatomical structure (Riege, 1971) and endocrine system (Lovely, Pagano, & Paolino, 1972) from those of early social isolation.

Various theories have been proposed on the effects of social isolation in the early stages of development. For example, while Einon and her colleagues concluded that early social isolation produced behavioural inhibition deficit that might be caused by missing the chance of experiencing contingency probability in infant stage (Einon, Morgan, & Sahakian, 1975; Einon & Morgan, 1977; Morgan, Einon, & Nicholas, 1975), Walsh & Cummins (1975), Baumal, De Feo, & Lal (1969) and De Feudis (1975) proposed the stimulus hyperreactivity theory (or hyperarousal theory) that a combination of activation of sensory systems and non-specific activation, or arousal, underlay some proportion of the differential brain effect, and an altered arousal state caused an altered level of sensory awareness and probability of engram storage. However these theories left the effects of isolation after maturity out of consideration. Only theory considering life span was Riege's atrophy hypothesis (1971) that isolation effects could occur even in juveniles and adults for systems that require input and use to maintain anatomical-physiological integrity.

Wright, Upton, & Marsden (1991) showed that 30-day isolation of the socially housed rats from weaning to 54 days old did not produce an anxiogenic profile on elevated X-maze. Similarly, Karakawa (1995 a) reported that both rats socially isolated after maturity (ISOL) and socially housed rats (SOC) learned mult FR 5 DRL 10 /LH 34 schedule, and that SOC rats did more lever-press responses than ISOL subjects and discussed that social isolation procedure after maturity did not affect the acquisition of mult FR DRL schedule per se. In other words, behavioural inhibition in the case of social isolation in early stage was not observed in ISOL rats.

On the other hand, Karakawa (1995 b) showed that ISOL and SOC rats learned FR 2 active avoidance task, and that SOC rats showed higher extinction resistance than ISOL rats. These results were similar to Karakawa (1995 a) in the point that social isolation after maturity had no effects on the acquisition of learning task. It was characteristic that ISOL rats moved around more frequently in habituation period than SOC rats. Dalrymple-Alford & Benton (1981) distinguished inspective exploratory that was directed towards discrete novel stimuli from non-directed aimless exploratory and concluded that the latter type was enhanced by early social isolation. This suggestion might be applicable to the result of Karakawa (1995 b). If this is true, the effects of social isolation after maturity should be interpreted from the hyperreactivity theory.

The present study had two purposes. The first was to reconfirm that the main deteriorious effect of social isolation after maturity was the increment of non-directed exploratory behaviour. Intellectual and complex learning tasks such as a Hebb-Williams maze and two-lever DRL schedule of reinforcement did not suit the experiment carried out to test whether the level of emotionality or arousal form the foundation of the performance. Open field test and FR 1 active avoidance were easy and were frequently used to examine the subject's fear and anxiety. The second was to demonstrate how to reduce the deficits derived from isolation procedure. Studies of early social isolation revealed that, though the full isolation produced permanent deficits, a brief social contact with conspecific extinguished them (Einon, Morgan, & Kibbler, 1978 ; Wade & Maier, 1986). Therefore it was expected that isolated rats with social contact would recover from the isolation syndrome.

## Method

**Subjects and housing conditions.** Thirty naive male albino rats of Wistar strain were used as subjects which were obtained from seven pregnant female rats bred in Hiroshima University. They were weaned at about 30 days old and reared in the same sex groups of 4-5 litters per cage. At 120 days, they were randomly assigned to the following three groups. The rats of ISOL 30 (n = 10) group were housed individually for thirty days before the beginning of experiment. Rats of ISOL 7 (n = 10) were housed in the same manner as ISOL 30 for 7 days, and SOC group (n = 10) housed in 5 rats per cage. The half of ISOL 30 and ISOL 7 experienced the partial social isolation (PISOL),

i. e., five rats of each group were regrouped for an hour per day for seven days before the start of experiment. The remaining half was always exposed to the total social isolation (ISOL), in which rat were allowed only to see, smell and hear each another without a contact. At least 60 % of each housing condition group consisted of the different litters. The size of individual cage was 15 x 30 x 24 cm, and that for group housing was 24 x 36 x 18 cm.

All the subjects were not disturbed except of routine cleaning and the experimental treatment for PI groups. The day / light cycle was 12 : 12 (7 : 00–19 : 00 on) and the temperature was maintained at about 24°C. Food and water were available ad lib in the home cage.

**Apparatus.** A wooden square open field (80 x 80 x 30 cm) with a clear Plexiglas ceiling was used for Open Field Test. Side walls were painted in white and the floor was black on which white lines was drawn 13.3 cm apart to mark out the field into 36 squares. The walls and a guillotine door of the start box jointed to the corner of open field were black. The open field was illuminated by fluorescent lights measured below 1 lx inside the start box and 460 lx on the floor of the open field.

Two identical shuttle boxes (35 x 40 x 24 cm) was used for active avoidance conditioning. Each box was made of Plexiglas and placed in a well-ventilated sound-attenuating room maintained at 25°C. It was divided into two compartments separated by a stainless steel partition with a semicircle-shaped opening measured 6.5 cm in diameter. The floor of each compartment consisted of 0.3 cm stainless steel rods spaced 2 cm apart, wired to a shock generator (Muromachi Kikai Co., Ltd, SGS-002 / T). An illumination (260 lx) provided by a 40-W bulb located 20 cm above the centre of the box served as the conditioned stimulus (CS) above the 77 lx background. The unconditioned stimulus (US) was a 0.5 mA shock. Rats' movements were monitored with the aid of two microswitches, each of which was attached to the root of the side wall. The background noise by an exhaust fan was 64 dB. Recording and the experimental control was carried out automatically by a personal computer (Panasonic MSX / R).

**Procedure.** For three days from 147 days of age, the rats were allowed to explore the start box of open field apparatus for 10 min. After this adaptation period, Open Field Test was done individually in successive 10 days. Rat was placed inside the start box for 10 min and then the guillotine door was raised. Rats could return to the start box as the guillotine door remained open. Daily testing was done randomly in respect of the housing condition. The time to emerge into the field was recorded in seconds with a stopwatch. If the rat did not emerge in 10 min the trial was terminated and a score of 600 sec was taken for the animal. The manual records of ambulation (squares traversed with fore paws) were taken for five consecutive minutes of each trial after rat's emergence. At the end of a trial, the rat was returned to the home cage and the floor and walls were cleansed with a mild disinfection solution. The order of testing was randomized with respect to housing condition.

From 160 days all subjects were tested for the acquisition and extinction of the escape / avoidance learning. Both acquisition and extinction phases consisted of 100 trials. Daily training session was 20 trials. In each trial of acquisition phase, the US was preceded by a 10 sec CS. Responding with a latency of less than 10 sec from CS onset enabled the rat to avoid shock. If the subjects' responses had not met the task demand (FR 1) by the end of the tone presentation, the US was administered together with the CS. Termination of both CS and US was contingent upon the crossing or the end of the 20 sec tone presentation. The procedure of extinction phase was identical to that of the acquisition phase except the US was not presented. The variables recorded were the latencies for the crossing and the number of crossings occurring during the average 1 min inter-trial interval. The order of testing was fixed with respect to housing conditions.

## Results

### Data analyses

The major concern of the present study was to examine whether the effects of isolation duration, if any, were diminished or attenuated by a brief socialization prior to the experimental treatments. To reveal this, several ANOVAs were first run on the variables as a function of the isolation duration (30-day isolation or 7-day isolation) and as a function of the degree of isolation (total isolation or partial isolation). In concrete, a four-way analysis of variance was carried out on the each score with isolation duration and socialization as the two main factors and minutes, days, or blocks as the repeated measures. But the factor of isolation duration had no effects on any variables and only the degree of isolation was effective as summarized in Table 1. So the factor of the isolation duration was pooled and one main factor with three levels (i. e., totally isolated ISOLs, partially isolated PISOLs and SOC) was used in the analyses described below. When appropriate, individual Newman-Keuls comparisons were run at the significance level of 5 %. Note that as two rats in SOC group not making at least 10 avoidance responses in the acquisition phase were excluded from the analyses on the performances of escape / avoidance learning, the data were eight for SOC group.

### Open Field Test

The emergence latency was transformed into the logarithmic scale, on which a two-way analysis of variance was carried out (Figure 1). The main effect of housing conditions was significant [ $F(2, 27) = 5.95, p < .01$ ] and Newman-Keuls comparisons showed that ISOLs rats took longer time to emerge from the start box than PISOLs and SOC subjects. PISOLs and SOC were alike. The main effect of day was significant [ $F(9, 243) = 3.13, p < .01$ ]. The interaction of housing conditions x day was not significant [ $F(18, 243) < 1$ ].

Figure 2 shows the mean number of ambulation for each minute of a 5-min daily trial totaled

Table 1 Summarized ANOVAs carried out on the scores with isolation duration and socialization

Variable	Source	<i>F</i> value	<i>df</i>	<i>p</i>
Emergence latency	A : Isolation duration	0.32	1/16	n.s.
	B : Socialization	10.37	1/16	< .01
	C : Day	2.58	4/64	< .01
	A x B	0.03	1/16	n.s.
	A x C	0.80	4/64	n.s.
	B x C	0.49	4/64	n.s.
	A x B x C	0.66	4/64	n.s.
Ambulations	A : Isolation duration	0.12	1/16	n.s.
	B : Socialization	1.55	1/16	n.s.
	C : Day	6.15	9/144	< .001
	D : Minute	14.28	4/64	< .001
	A x B	0.01	1/16	n.s.
	A x C	0.79	9/144	n.s.
	B x C	1.52	9/144	n.s.
	A x B x C	0.77	9/144	n.s.
	A x D	0.75	4/64	n.s.
	B x D	4.64	4/64	< .01
	A x B x D	0.09	4/64	n.s.
	C x D	1.24	36/576	n.s.
	A x C x D	0.76	36/576	n.s.
	B x C x D	0.79	36/576	n.s.
A x B x C x D	0.78	36/576	n.s.	
Avoidance responses in acquisition phase	A : Isolation duration	0.02	1/16	n.s.
	B : Socialization	0.05	1/16	n.s.
	C : Blocks	42.76	4/64	< .001
	A x B	0.93	1/16	n.s.
	A x C	0.43	4/64	n.s.
	B x C	1.50	4/64	n.s.
	A x B x C	1.71	4/64	n.s.
Avoidance responses in extinction phase	A : Isolation duration	0.29	1/16	n.s.
	B : Socialization	0.25	1/16	n.s.
	C : Blocks	11.43	4/64	< .001
	A x B	0.56	1/16	n.s.
	A x C	1.81	4/64	n.s.
	B x C	0.71	4/64	n.s.
	A x B x C	0.85	4/64	n.s.

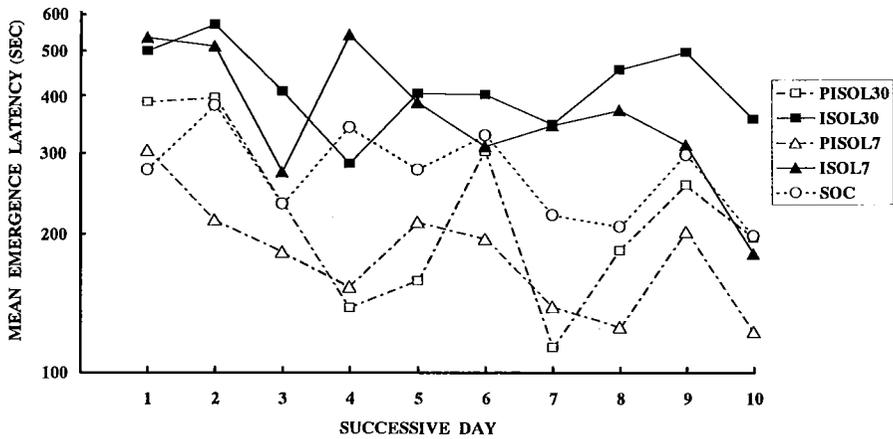


Figure 1 Mean emergence latency from start-box into open filed. The vertical axis represents the logarithmic scale.

over the 10 days. The main effect of housing conditions was not significant [ $F(2, 27) = 1.33$ ]. The main effect of day [ $F(4, 108) = 25.03, p < .0001$ ] and the interaction of housing conditions  $\times$  min [ $F(8, 108) = 3.01, p < .01$ ] were significant. Newman-Keuls comparisons showed that ISOLs moved fewer for the first one minute than PISOLs and SOC subjects. There were no differences between PISOLs and SOC, and among groups in later four minutes.

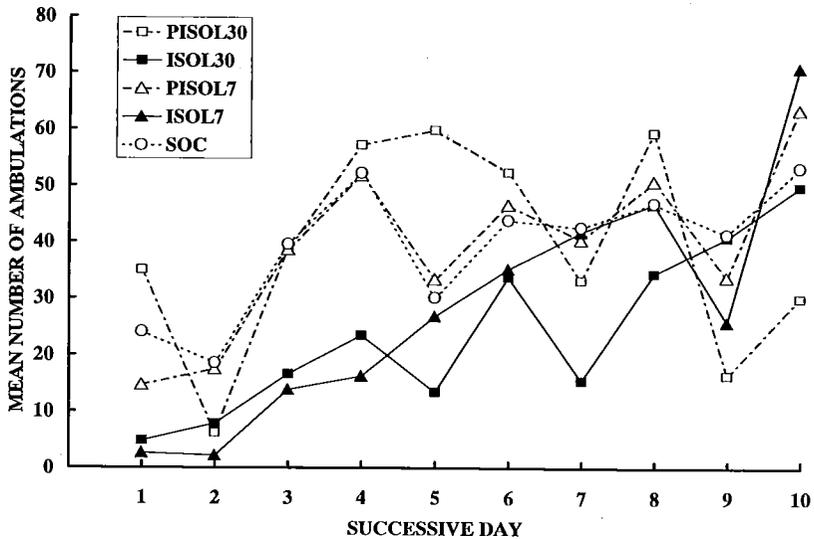


Figure 2 Mean number of ambulation (locomotor activity) for each minute of a 5-min daily trial totaled over the 10 days.

### Escape / Avoidance training

The mean number of avoidance responses during acquisition and extinction phase for the five housing condition groups are presented by 20-trial blocks in Figure 3. The number of avoidance in acquisition did not differ among SOC, PISOLs, and ISOLs [ $F(2, 25) < 1$ ]. The main effect of housing conditions [ $F(2, 27) = 1.33$ ] and the interaction of housing conditions  $\times$  min [ $F(8, 100) = 1.72$ ] were not significant. The main effect of blocks [ $F(4, 100) = 45.45, p < .0001$ ].

In the extinction phase, only the main effect of blocks was significant [ $F(4, 100) = 16.60, p < .0001$ ]. The other main effect [ $F(2, 25) < 1$ ] and the interaction [ $F(8, 100) < 1$ ] were insignificant.

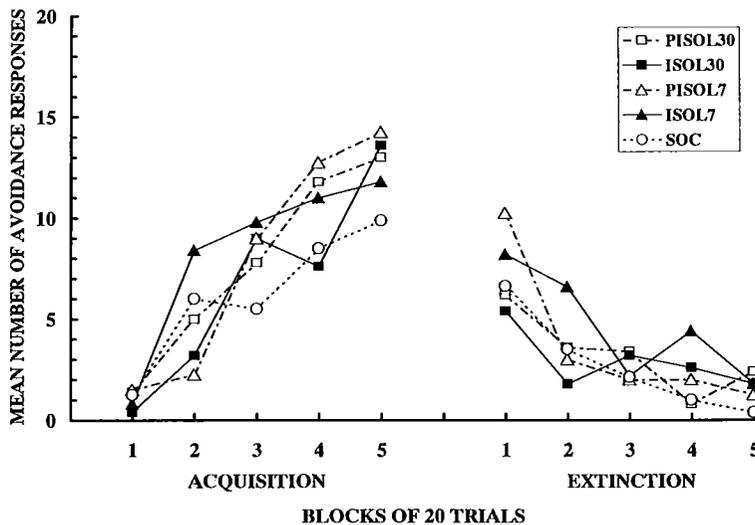


Figure 3 The mean number of avoidance responses per 20-trial block during acquisition and extinction phase of FR 1 active avoidance task.

### Discussion

The purpose of this experiment was to examine the nature of the adult rats' isolation-induced changes and how to reduce those effects.

In Open Filed Test, fully isolated adult rats hesitated to move into open filed and their ambulation score for the first one minute after emergence was lower than group-housed rats and partially isolated group. Supposed that isolated animal's hyperactivity was due to the increment of their non-directed exploratory and the decrement of inspective exploratory (Dalrymple-Alford & Benton, 1981), the isolated should consistently move around open field through five minutes. The contradictory result might be caused by the structure of open field. Because the situation employed in

this experiment was free open field that had a start box and let rats explore as they pleased, they could return from the field to the start box and stay there. In other words, rats did not have to dare to explore the field when they felt strong anxiety. Observation supported that the fully isolated was excessive afraid of novel situations. ISOLs had a tendency that they move along the walls of open field and rarely walked into the center area while SOC and PISOL rats frequently did so. Forced Open Field Test without a start box would revealed the isolated rats' fearfulness more clearly.

In Escape / Avoidance Test, isolation after maturity did not affect the acquisition of FR 1 active avoidance task. The result could be interpreted in two different ways. One is that isolated adults rats actually learned the avoidance task as easily as SOC and PISOLs group. Another possibility is that no difference is ostensible. Even if ISOLs were more anxious than SOC and PISOLs, their accidental hyperactive movement to the other side of shuttle-box would be reinforced more effectively. In addition, isolation effects might be diminished during Open Field Test.

Though the nature of isolation after maturity remained ambiguous, isolated adults actually suffer some kinds of deficits unlike Wright, Upton, & Marsden (1991) and Karakawa (1995 a). Further researches of isolation effects should be focused on arousal systems, attention and emotionality.

In contrast, the effect of resocialization was dramatic. The rats of ISOLs group rapidly recovered from isolation effects by experiencing 1-hour social contact with conspecifics during the isolation period, but living otherwise in isolation. This procedure was identical to that of Eimon et al. (1978) who allowed the isolated infant rats body contacts. The magnitude of resocialization effect, however, was different. Though the performances of PISOL were between SOC and ISOL in the experiments of Eimon et al. (1978) and Wade & Maier (1986), PISOL rats did as well as SOC in this experiment. The difference might depend upon the effects of isolation before and after maturity. As mentioned above, any disturbance was observed in the complex learning tasks by isolation in adults.

Further researches are necessary to examine other easy methods to improve the isolation effects. The handling, for example, is a routine work before experiment. If handling is effective to modify the isolation effects, investigators can neglect the influences deprived from a isolation procedure used in usual animal experiments.

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## Effects of a brief social contact upon open field behaviour and FR 1 escape / avoidance acquisition in socially isolated adult rats

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Abstract. A 2 x 2 factorial design with a hanging control group was employed to examine whether social isolation after maturity would affect the open field test and the FR 1 shuttle-box avoidance and whether a brief social contact would reduce those effects. The factor of social duration had no effect on all the independent variables of both tests. The another factor of social contact, on the other hand, had remarkable influences. The rats of PISOL 30 and PSIOL 7 (PISOLs) experiencing 1-hour social contact a day emerged into open field in shorter time than ISOL 30 and ISOL 7 (ISOLs). The performances of PISOLs were equal to those of SOC rats without social isolation experience. Concerning with ambulation scores in open field, the rats of PISOLs and SOC groups moved more than ISOLs during the first minute after their emergence into open field. There was no significant difference in the number of FR 1 avoidance response during the acquisition phase among SOC, PISOLs and ISOLs. These results had three interesting implications ; (a) isolated adult rats seemed to be more anxious for novel stimuli than group-housed rats and partially isolated rats, (b) isolation did not exert any influence on FR 1 shuttle-box avoidance learning task, which was dissimilar to the effects of social isolation in infant, and (c) a brief contact with conspecifics remove the detelious influences of social isolation.

Key Words : open field, shuttle-box avoidance, social isolation, socialization, adult rat