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Psychosocial Hypertension in Members of a Wistar Rat Colony[1] (38063)

NATALIE ALEXANDER

Department of Medicine, Clinical Pharmacology Section, University of Southern California, School of Medicine, 2025 Zonal Avenue, Los Angeles, California 90033

A blood pressure study by Bernardis and Skelton (1) indicated that rats housed 3 per cage eventually developed greater arterial pressure elevation than those housed 1 per cage. Apparently this suggested model for rat hypertension was not further investigated except in a limited manner by Dahl (2) who discussed an unsuccessful attempt to produce hypertension in rats by crowding.

The purpose of this study was to determine if aggregated rats became hypertensive and if so, whether the hypertension was associated in individual rats with any particular type of behavior evoked by the conditions of aggregation. The general protocol included a period of isolation (3), after which rats were aggregated in physical conditions similar to those used to induce hypertension in aggregated mice, namely, a single cage or an interconnecting system of cages (4).

Methods. Isolation. Two sets of pure line Wistar rats were received in the laboratory, 2 weeks apart, when each set was 21 days of age. Fifty-two rats were in Set 1 and 50 in Set 2. Each set consisted of members of 6–8 litters and an equal number of males and females. Rats were housed individually in an opaque mouse cage with a wire top, 12.5 × 4.5 × 5.5 in. Cages were hung on a rack and rats were isolated physically and visually from each other. Two racks, each with 1 set of rats, were placed in the same room of the vivarium. When rats were 4–5 months of age, a number was tatooed at the base of the tail for individual identification and pressure measurements were started.

Blood Pressure. The tail cuff method for systolic pressure measurement described for mice (4) was adapted for rats. The occlusion cuff had the same dimensions recommended by Pfeffer *et al.* (5) for rats of the same body weight and strain. Pressure measurements were made every other week and postaggregation pressures were compared to an average control value of 4 measurements obtained during the last 2 mo of isolation. Pressures were also measured 24 or 48 hr after the start of aggregation.

Physical Conditions of Aggregation. Rats were aggregated either in a system of interconnecting cages or in a single cage (see below). At first, only Set 1 rats were aggregated, then after 3 weeks, when construction of additional cages was completed, an equal number of Set 2 rats were mixed with Set 1 rats for the remainder of the study. Figure 1 shows the time sequence of isolation and aggregation of both sets of rats and indicates the number of rats of each sex assigned to the 2 physical conditions of aggregation or to continued isolation. Rats were assigned placement by random selection of numbers; those not selected remained isolated.

For clarity of presentation, the 2 conditions of aggregation and the results obtained with each will be described separately although both experiments were carried out simultaneously and in the same room of the vivarium that housed the isolated rats.

Wheels. A wheel consisted of 6 stainless steel cages surrounding a central feeding area. See diagram, Fig. 2. Each cage had a wire top, solid slides and bottom and was 12 × 9 × 9 in. The central hexagonal area

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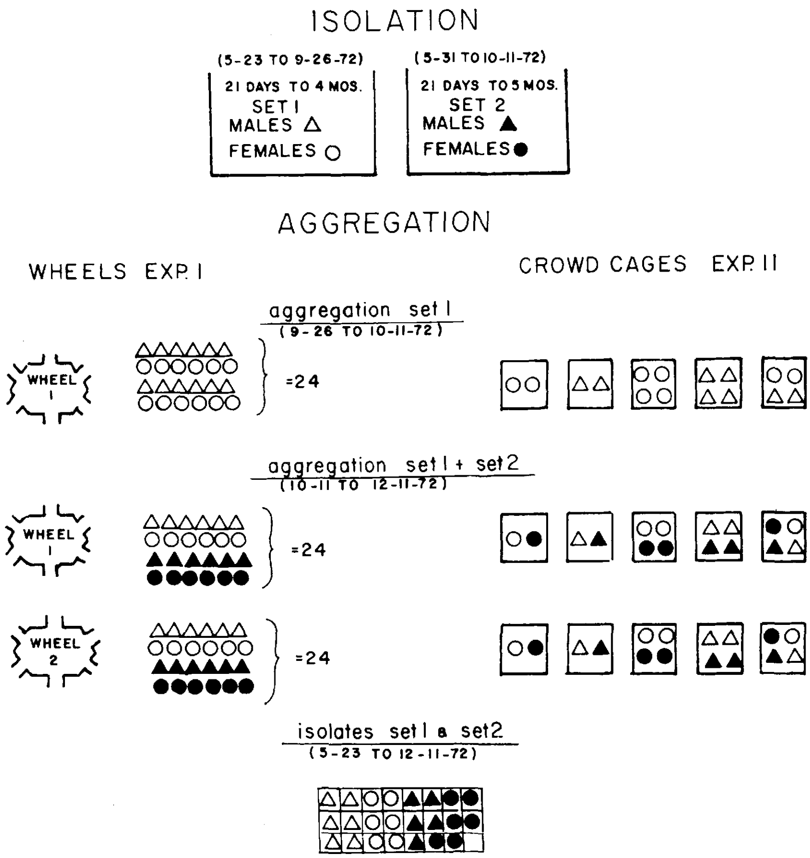

FIG. 1. Time sequence and distribution of rats by Set and sex for isolation and aggregation.

was 16 in. across with 18 in. high plastic walls from which a wire food hopper was suspended. A hole in each wall admitted a spout from a water bottle attached to the outside of the wall. Cages were connected to each other and to the center area by 2.5 in. diameter plastic tunnels which allow only one rat to pass at a time. This forces encounters so that one rat has to back off.

Crowd Cages. These were single 12 × 9 × 9 in. cages like those used in the wheel (see above). Rats were aggregated in crowd cages in 1 of 3 ways. (a) in pairs of the same sex, (b) in groups of 4 of the same, or (c) mixed sex, see Fig. 1.

Observations. Following aggregation, rats were observed twice a day, 5 days a week. The times of observation were varied and the duration ranged from about 5–30 min depending on the general activity of the rats. Specific types of behavior were noted and recorded. We looked especially for signs of aggressive behavior by individual rats, i.e., fighting, presenting flank and assuming the boxing position (6). Periodically, the number of tail bites on each rat was recorded.

Results. Experiment I. Wheel Aggregation. Twenty-four hours after the start of aggregation of Set I rats, 41% of wheel rats had a 15 mm Hg or more increase above the average isolation pressure.[2] A

[2] An increase of 15 mm Hg was approximately twice the S.D. of the mean of isolation systolic pressures which were 135 ± 7 mm Hg for all males and 128 ± 8 mm Hg for all females. This sex difference was statistically significant, $p < .001$.

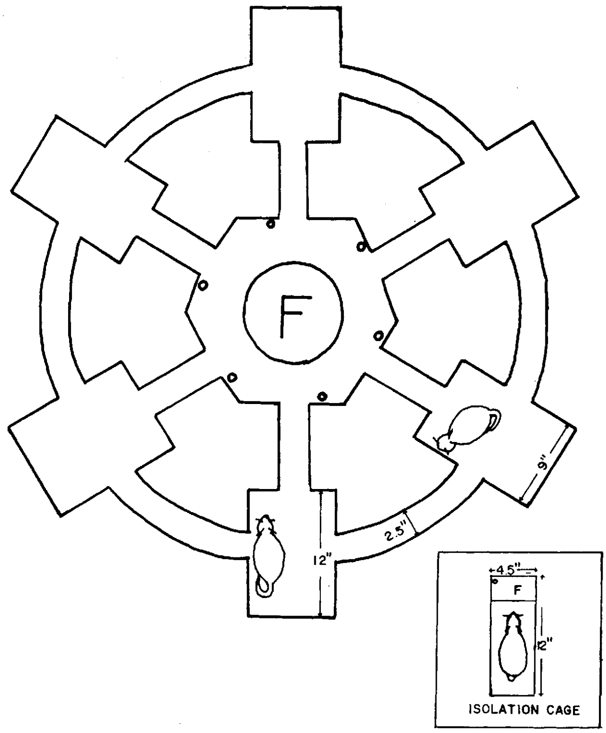

FIG. 2. Diagram of wheel arrangement for aggregating rats. F = food hopper and O = water spouts. Size of rat in relation to cage size is indicated. *Insert:* isolation cage 12 × 4.5 × 5.5 in., shown for comparison to wheel.

few male rats had body or tail bites, indicating that fighting had occurred, yet wheel rats were found piled on top of each other in several cages. During the next 2 weeks the incidence of arterial pressure increase was less and very little fighting was observed.

At this time Set 2 rats were aggregated with Set 1 (see Methods and Fig. 1). Within an hour after aggregation of Set 2 with Set 1 rats in wheels, fighting started with considerable intensity. By 2 hr, numerous males in Wheel 1 had bloody wounds; fighting in Wheel 2 was less intense and fewer males had wounds than in Wheel 1. The incidence of at least a 15 mm Hg rise was no greater than when fighting was less severe among Set 1 rats only; it was 41% among Set 2 and 27% among Set 1 rats 48 hr after both sets were aggregated in wheels.

Pressures obtained during 7–8 weeks of aggregation were averaged for each rat and compared to its average isolation value. Among wheel rats more males than females showed an average increase of 15 mm Hg or more. In 9 of 23 (37%) wheel males the range of average increase was 15–46 mm Hg, average 21 mm Hg, and in 7 of 24 (29%) wheel females the range of average increase was 15–27 mm Hg, average 22 mm Hg. Their weekly values are graphed in Fig. 3 along with pressures obtained on rats kept in isolation during the same period. Note the increased S.D. around means of aggregated rats; some had pressures of more than 170 mm Hg and one had values over 190 mm Hg.

Weekly arterial pressures of all wheel rats were divided into 4 groups according to Set and sex and the average increase for the total period of aggregation, ranging from 7 to 16 mm Hg above control isolation pressure, attained statistical signifi-

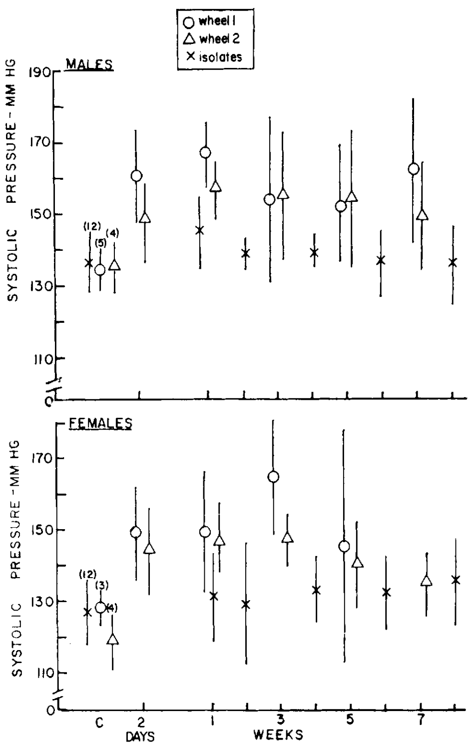

FIG. 3. Average systolic pressure, Mean ± S.D., of 9 wheel males and 7 wheel females that had, during 7 wk of aggregation, an average pressure increase of at least 15 mm Hg. Pressures of 12 isolated males and 12 isolated females are shown for comparison.

cance in 3 of the 4 groups in each wheel.[3] The arterial pressure difference between males and females found during isolation (see footnote 2) was maintained during aggregation in Wheel 2 but not in Wheel 1.[3] By contrast with wheel rats, no pressure increase occurred in 3 of the 4 isolated groups of rats whose pressures were averaged for the same period.[4]

Unexpectedly, when rats were aggregated in wheels, 1 male rat became dominant. Dominant or subdominant behavior among wheel aggregated males included fighting behavior although the reverse was not the case. The major dominant was readily identified by the following behavior. (1) it was in the center area at almost any time of the day, whereas other rats were scattered about in the surrounding 6 cages; (2) it chased other rats out of the center when they attempted to eat and drink; (3) it often was seen presenting flank and attacking other males; (4) it frequently put its head inside a tunnel to screech at another rat attempting to enter the center area through the tunnel. A rat was identified as subdominant or as a rival dominant if it was observed to fight with the dominant or with other rats in the center area more than once.

Twenty-four hours after aggregating Set 1 and Set 2 rats in Wheel 2, a male rat, #17 (Set 1), was seen alone in the center area of Wheel 2. Thereafter, #17 was seen in the center consistently. He often slept there during the day; all male rats except #17 lost weight during the period of aggregation because #17 restricted their access to food. This dominant rat's pressure was only temporarily elevated, i.e., during the first 2 weeks of aggregation, as shown at the top of **Fig. 4.**

The dominant male in Wheel 1, #101 (Set 2), was much more active than #17, i.e. #101 patrolled the center area with great frequency, lost one eye in a fight, and was almost never seen to sleep in the center. More fights occurred in Wheel 1 than in 2. During the 8th week there was a sudden new burst of fighting in Wheel 1 that lasted several days. During those days, #101 and #5 were often seen fighting to-

[3] The average pressure increases during 7 weeks of wheel aggregation compared to isolation pressures were: Wheel 1: Set 2, males, 16 mm Hg ($p < .01$) and females, 11 mm Hg ($p < .02$) and Set 1 females, 9 mm Hg ($p < .001$); Wheel 2, Set 1 males, 7 mm Hg ($p < .05$) and females 14 mm Hg ($p < .001$) and Set 2 males, 9 mm Hg ($p < .01$). Pressure increases of 9 and 6 mm Hg were not statistically significant for males, Set 1, wheel 1 and females Set 2, wheel 2, respectively. Combined Set 1 and 2 average aggregated pressures were: Wheel 1, males, 144 ± 26, mm Hg, females, 139 ± 13 mm Hg, p = n.s.; Wheel 2, males, 144 ± 13, females, 136 ± 13, $p < .05$.

[4] Pressures of isolated Set 1 and 2 males and isolated Set 2 females averaged for the same period as wheel aggregated rats did not show any increase; a 6 mm Hg average rise for isolated Set 1 females was not statistically significant.

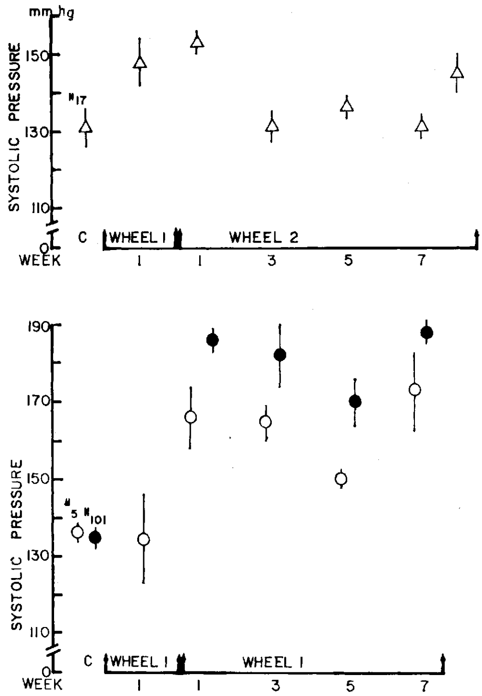

FIG. 4. Average systolic pressures of 3 dominant rats during 7 wks of wheel aggregation.

gether, until finally #5 emerged the new dominant rat. Figure 4, bottom, shows that both #101 and #5 were hypertensive rats.

In addition to the 3 dominant types of rats already mentioned, 7 other wheel males were identified as subdominant. Ely showed that among mice, the number of tail bites was inversely related to the frequency of dominant behavior (7) and we found a similar relationship in wheel aggregated male rats. Thus the 10 rats behaviorally identified as dominant types had, at the end of 7 weeks of aggregation, an average of 3 (range 0–6) tail bites, compared to an average of 7 (range 1–12) among the other 13 wheel males ($p < .02$). We then divided all wheel males into 2 groups on the basis of number of tail bites and compared their arterial pressures. Fifteen rats had the least number of bites, 0–5, while 8 had 6–12; the former were considered dominant types. Table I shows that the frequency of pressure increase was the same in both groups but among those that developed hypertension the magnitude of pressure increase was significantly greater ($p < .05$) in those with the least number of tail bites.

Experiment II: Crowd Cages. Sixteen Set 1 rats were aggregated in crowd cages in pairs or groups, see Fig. 1. Twenty-four hours after the start of aggregation, 25% of crowd cage rats, in contrast to 41% of wheel aggregated Set 1 rats, had a 15 mm Hg or more increase above the average isolation pressure (see footnote 2). Unlike wheel rats, no bites were found on crowd cage rats at 24 hr.

Set 2 rats were mixed with Set 1 rats and Fig. 1 shows the arrangement of rats in crowd cages. Fighting was infrequent and

TABLE I. Relationship of Arterial Pressure Increase to Dominance and Subdominance as Indicated by Least Number of Tail Bites Among Wheel Male Rats.

Type	No. of tail bites	No. of male rats*	No. (%) without pressure increase	Arterial pressure increase** No. of rats	Mean ± S.D.*** mm Hg
Dom. or Subdom.	0–5	15	4 (27%)	11	18 ± 11
Others	6–12	8	2 (25%)	6	8 ± 5
					$p = < .05$

* One male died after 2 weeks; this left a total of 23 males aggregated in both wheels for 7 weeks.

** Increase is average change during 7 weeks of aggregation from the average control isolation pressure.

*** If those without any pressure increase were included, the average increases were 13 ± 12 mm Hg and 6 ± 5 mm Hg for those with 0–5 and 6–12 tail bites, respectively, and the difference was not statistically significant.

mild among crowd cage groups during the first couple of hours after aggregation of both sets, and thereafter no fighting occurred and no tail or body bites were found on crowd cage rats. Still the incidence of a 15 mm Hg or more pressure increase 24 hr after being aggregated in crowd cages was about the same as in the wheels (see above), namely, 44% of Set 2 and 38% of Set 1 crowd cage rats.

Pressures obtained during 5–7 weeks of crowd cage aggregation were averaged for each rat and compared to its average isolation value. Females had a higher incidence of a 15 mm Hg or more pressure rise than males, i.e., 6 of 16 (38%) females and 4 of 16 (25%) males. When, however, aggregated pressures for all crowd cage rats were compared by sex, no significant difference was found. Taken as groups rather than as individual rats, 4 of 10 (40%) groups of crowd cage rats had average pressure increases ranging from 12 to 29 mm Hg, a statistically significant increase compared to the group average isolation pressure. Table II shows the pressures of the 4 groups.

Discussion. Rats were aggregated to create an atmosphere of continuous psychosocial stimuli which could activate the autonomic nervous and endocrine systems of the animals with sufficient intensity and frequency to evoke a measureable cardiovascular change. The 2 general types of physical environments used to aggregate mice and induce hypertension (4) were applied to rats in the present study. The results showed that some rats developed large pressure increases and most rats developed some elevation of arterial pressure under these conditions. We attempted to relate the development of hypertension in individual rats as well as in groups to specific types of behavior. We found that neither dominance nor fighting behavior was the *sine qua non* for development of hypertension since, (1) females became hypertensive without fighting or displaying any of the criteria for dominance, and (2) the crowd cage rats, males and females, became hypertensive without participating in or directly observing fighting and (3) the frequency of pressure rise in wheel aggregated males was the same among dominant and nondominant types (based on least number of tail bites). However, among wheel males that developed hypertension dominant types had the largest pressure increases (Table I). Other kinds of specific behavior by individual rats, e.g., maternal behavior, i.e., degree to which young were cared for and protected by a female, or cannibalism of young by certain males and females or, behavior toward humans, i.e., timidity or aggressiveness, apparently were unrelated to hypertension, at least not in any clear cut manner.

The wheel environment was designed to cause social disorganization, i.e. to prevent the establishment of territories and so arouse fear and anxiety by thwarting stereotyped behavior that accompanies territoriality. This apparently was accomplished to some extent since newborn pups were usually neglected and often eaten by both males and females and the death rate was high among pregnant rats, and preliminary findings show that adrenals were enlarged in wheel aggregated rats. Unexpectedly, however, rats learned rapidly that territory could be established in the center area of a wheel in spite of its multiple entrances. This

TABLE II. Average Systolic Pressure (Mean ± S.D.) of 4 Groups During Isolation and During 5–7 Weeks of Crowd Cage Aggregation.

	Isolation	Aggregation	p
Group 1: Mixed Sex			
Females (2)	126 ± 5	155 ± 12	<.001
Males (2)	136 ± 8	150 ± 12	<.01
Group 2: Females (4)	127 ± 7	139 ± 9	<.001
Group 3: Females (4)	131 ± 9	143 ± 15	<.02
Group 4: Males (2)	135 ± 5	152 ± 16	<.02

degree of social orderliness may have minimized the frequency of occurrence and magnitude of hypertension. In crowd cages no fighting occurred and newborn pups of mixed sex groups were always cared for and raised to weaning in spite of the small available space.

With the change from isolation to aggregation, the area available to each rat in single crowd cages was diminished by one-half (groups of 4) or stayed the same (pairs), and in the wheels, cage space per rat was diminished by one half but tunnel and center area space was additional (see Fig. 2). This was similar to the space change used to induce high arterial pressure among aggregated mice except more total mice were present in a single cage or wheel environment (4). In those studies, a single mouse apparently did not become dominant whereas a single rat did in the present study. Body size relative to physical dimensions of each environment was much greater for the rats than for mice. The large size of the rat in relation to cage dimensions may account for the facts that a single rat could establish dominance or territory in the wheel and that crowd cage rats did not fight.

Summary. This study was an attempt to induce hypertension in rats by the continuous psychosocial stimuli caused by aggregation. Wistar rats of both sexes were isolated from weaning to 4–5 mo of age. Tail cuff pressures were obtained every other week during the last 2 mo of isolation and up to 8 weeks of aggregation. Two types of physical conditions were used for aggregation: (1) 6 interconnecting cages in a wheel-like arrangement around a central eating and drinking area; (2) a single cage. Two wheels were used each with 24 rats, 12 males and 12 females and 10 single cages, each with 2 or 4 rats of the same sex. Other rats of both sexes remained isolated as controls. Approximately one-third of all aggregated rats of both sexes showed a 15 mm Hg or more average increase of systolic pressure during aggregation; other aggregated rats showed less average pressure rise. Rats in single cages did not fight nor did wheel aggregated females; nevertheless, arterial pressure increased among these rats. Among wheel aggregated males that developed hypertension, those classified as dominant types, by the least number of tail bites, had the largest pressure increases. The frequency of pressure increase was the same in dominant and nondominant wheel males.

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