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Effects of social isolation and crowding on sexual behaviour in the rat (*Rattus norvegicus*)

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ABSTRACT. *Effects of social isolation and crowding on sexual behaviour in the rat* (Rattus norvegicus).- The effects of social isolation and crowding on the sexual behaviour of Sprague Dawley male and female rats (*Rattus norvegicus*) were investigated. The major housing condition effect was found among social isolates. A significant decrease in their non-copulatory activity was observed in both genders when compared with socially reared animals, but only a similar tendency appeared with respect to their copulatory activity. No clear effects of crowding on the animal's sexual behaviour was found. The importance of social experience during development for a complete sexual display in the adulthood is discussed. The absence of noticeable effects of crowding upon specific sexual patterns, and the influence of high population density upon several physiological systems are also discussed.

KEY WORDS. Rat, Social isolation, Crowding, Sexual behaviour

Introduction

The major role that social experience plays in sexual behaviour, has been widely demonstrated in a variety of mammalian species (Valenstein et al., 1955; Harlow, 1962; Gruendel & Arnold, 1969; Anderson et al., 1972). Among rodents, the effects of social isolation appear to be dependent upon the species concerned. Thus, while a facilitating effect on the male mouse sexual behaviour has been reported (De Catanzaro & Gorzalca, 1979a, 1979b; Gorzalca & De Catanzaro 1979; De Catanzaro & Gorzalca, 1980), an opposite effect has been observed in hamsters, gerbils and, particularly, in the rat (De Catanzaro & Gorzalca, 1979a). Generally speaking, a normal social experience during development seems to be required for the expression of an adequate sexual behaviour in the adult rat. However, it has been reported (Hole et al., 1986) that only when a total isolation treatment (physical and visual and/or olfactory) is imposed, a significant degree of sexual impairment is achieved in the laboratory rat. Other data indicate that a prolonged isolation treatment of adult rats, which does not exclude the access to olfactory and auditive stimuli, causes a disruption in the male's sexual activity, although an almost normal sexual behaviour was found in the social isolates after repeated testing (Chambers, 1982). The question of which kind of isolation treatment to choose, and to which extent it causes sexual impairment appears to call for a further clarification. Moreover, a different approach to this kind of work is required, namely in studying the sexual behaviour of pairs of animals, both bred under the same social conditions, which may provide interesting information. On the other hand, most data about the influence of high population density on rodent reproduction refer to physiological factors rather than to specific sexual performances. Thus, according to Christian's model, which includes a number of endocrine systems, crowding appears to inhibit reproductive function, mainly through inhibition of gonadotropin secretion. In addition, several negative effects of crowding on gestation and upon the survival and growth of the next generations are included in that model (Gray, 1971; Christian, 1979). The present work contributes information about the effects of crowding on specific sexual behaviour patterns in the rat. Unlike other authors (De Catanzaro & Gorzalca, 1979a; Chambers, 1982; Hole et al., 1986) we tested the sexual behaviour of pairs of animals, both reared under the same social conditions. In fact, the active role of the female in the course of sexual interactions (McClintock & Adler, 1978; Madlafousek & Hlink, 1983) must be taken into account, and this is a matter that, to our minds, has not been given sufficient consideration in this kind of study. By using a female reared under the same social conditions as its partner, we studied the efffects of isolation and crowding on sexual behaviour from the point of view of adaptation.

Material and Methods

Animals and housing conditions

A total of 88 male and female Sprague-Dawley rats were used. The parental generation was supplied by "Panlab", and the experimental animals were bred in our laboratory. The animals were kept in a room at approximately 22°C with a 12 h reversed light-dark cycle (20:00-08:00 hours light on). Food and water were supplied *ad libitum.* Differential housing was introduced at weaning (22 days of age); the experimental groups were as follows: social isolated males (SIM, N=14) and social isolated females (SIF, N=14), housed in individual 24x24x15 cm cages; control males (CM, N=15) and control females (CF, N=15) housed 4-6 animals per cage; and crowded males (CRM, N=15) and crowded females (CRF, N=15) housed 15 animals per cage. Socially reared animals (control and crowded rats) were housed in 48x24x15 cm standard laboratory cages. Individually housed rats could receive several stimuli (other than tactile) from the other animals living in the same room. Thus, the isolation was considered as being "social".

Procedure

The sexual activity of sexually-naive male and female rats, of about 120 days of age, both reared under the same housing conditions, was tested. Ten-min tests took place in an open-field with a diameter of 75 cm, lit by a red light. Continous background noise was provided by an electric fan. Only receptive females whose natural oestrous had been verified by vaginal smears were used. In addition, the occurrence of dart-hopping responses was also checked to ensure sexual receptivity (Chambers, 1982). In this way, we avoided any manipulation (hormonal-induced oestrous) which otherwise might have disguised the potential physiological and behavioural modifications induced by the social treatments. A single test was performed for each pair of animals. Each test was commenced by placing the two animals in two opposite sites of the open-field. The time elapsed until the first physical contact with sexual cues took place (Emergence Time, ET) was recorded. Several criteria from previous studies (Madlafousek & Hlink, 1976) were taken into account to define the sexual postures. Two well characterized kinds of sexual performances were distinguished in the present experimental design, copulatory patterns proper, consisting of motor reflexes with a clear genetic dependence, and non-copulatory behaviours appearing during the precopulatory courtship phase. These latter approaching-recognizing performances, involving a number of visual, olfactory and tactile interactions have, in turn, a major dependence on the degree of maturation of several sensory systems. From this point of view, they can be considered as a whole, in the hypothesis that the experimental treatments used in this work (which affect the physical and social environment of the animals) may have a differential influence on these non-consummatory performances as compared to those consummatory patterns with major genetic dependence. Those non- consummatory behaviours involving a series of previous attractive-soliciting patterns were then defined as "non copulatory postures" (NCP) and included: - anogenital sniffing: olfactory exploration of the partner's anogenital area; - body licking: an animal licks its own back and flanks; - genital licking: which, in the case of the male normally occurs inmediately after copulation; - licking of the partner's body: one of the two animals licks the partner's head, back or flanks; - chasing: a typically precopulatory male pattern, aimed at seizing and inmobilizing of the female; - dart-hopping: a typical "soliciting" behaviour showed by the receptive female, involving a rapid locomotion which includes a sequence of hops and abrupt halts. Other patterns involving a well-characterized motor integration aimed at a typically copulatory behaviour were defined as "copulatory postures" (CP) and included: - lordosis: the characteristic posture of a sexually receptive female during copulation, involving a concave arching of the back; - attempted mount:the male clasps the female's back area, but the female shows no lordotic response and the mount is not accomplished; - mount: the male, in a well-oriented position, mounts the female, while this responds with a lordosis. Finally, females' vocalizations during the test were recorded. As noted above, the CP were considered as a whole for statistical analysis, and so was the overall NCP. The other variables studied were the ET in males and the vocalization task in females.

Results

The effects of the housing conditions on copulatory and non-copulatory postures were evaluated by a set of four "one-way" analysis of variance. The analysis of NCP revealed significant effects of social treatments both in males ($F_{(2.41)}=7.10$, $p<0.005$) and in females ($F_{(2.41)}=4.43$, $p<0.05$). Post hoc individual comparisons of means were done by the Scheffé test. For the males, these tests revealed that the social condition affected the social isolates, which showed a significant decrease in their NCP compared to the controls ($p<0.005$). No significant difference was found between control and crowded animals (fig. 1). Among female rats, a similar social isolation effect appeared. In this case, the multiple-comparison test revealed that the decrease in NCP was significant when social isolates were compared with the crowded females ($p<0.05$). No other significant difference was found in this set of tests (fig. 1). The statistical analysis of CP within each sex revealed no significant effect of social treatments. However, a tendency to show decreased copulatory activity was observed in the SIM group (fig. 2). The effects of the housing conditions upon the emergence time were evaluated by $\chi2$ Contingency tests. Three $\chi2$ values were calculated for comparisons between each two male experimental groups. The tests revealed a unique significant difference affecting the SIM group, which showed increased ET compared to the CM group ($\chi^2_{(df=1)}=3.94$, $p<0.05$) (table I). The SIF group showed more vocalizations (4.5±1.5) than the other female groups (CF, 1.5±0.6 and CRF, 1.5 ± 0.7), althought no significant difference was revealed by the "one-way" analysis of variance.

TABLE I. Male groups emergence time. SIM: socially isolated males; CM: control males: CRM: crowded males. * significantly different from control group, $p<0.05$.

[Tiempos de emergencia de los grupos de machos. SIM: machos socialmente aislados; CM: machos controles; CRM: machos hacinados. * diferencias significativas con respecto al grupo control, p<0,05.]

	Emergence Time	
	0.4-18.8 sec	18.8-64.8 sec.
SIM	5	8 (*)
CM	10	3
CRM	9	4

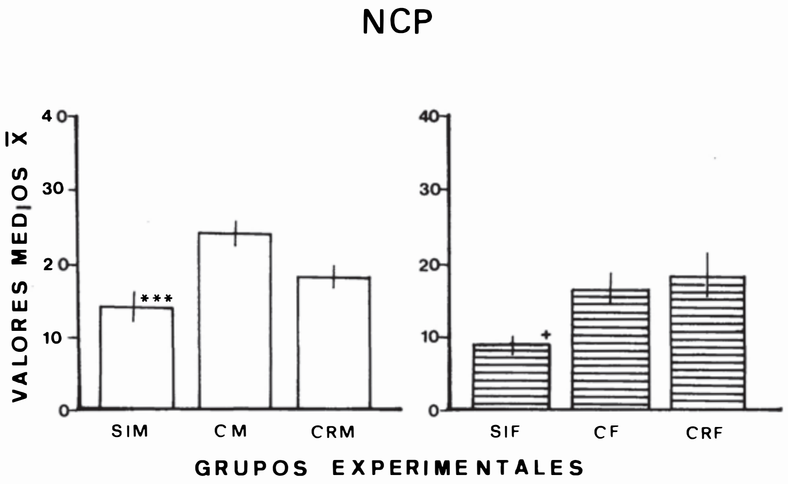

FIGURE 1. Non copulatory postures (NCP). Values are means±SE. SIM: socially isolated males; CM: control males; CRM: crowded males; SIF: socially isolated females; CF: control females; CRF: crowded females. *** significantly different from control males, p<0.005. + significantly different from crowded females, p<0.05.

[Posturas no copulatorias (NCP). Los valores son medias ±EE). SIM: machos socialmente aislados; CM:machos controles; CRM: machos hacinados; SIF: hembras socialmente hacinadas; CF: hembras controles; CRF: hembras hacinadas. *** diferencias significativas con respecto a los machos controles, p<0,005. + diferencias significativas con respecto a las hembras hacinadas, p<0,05.]

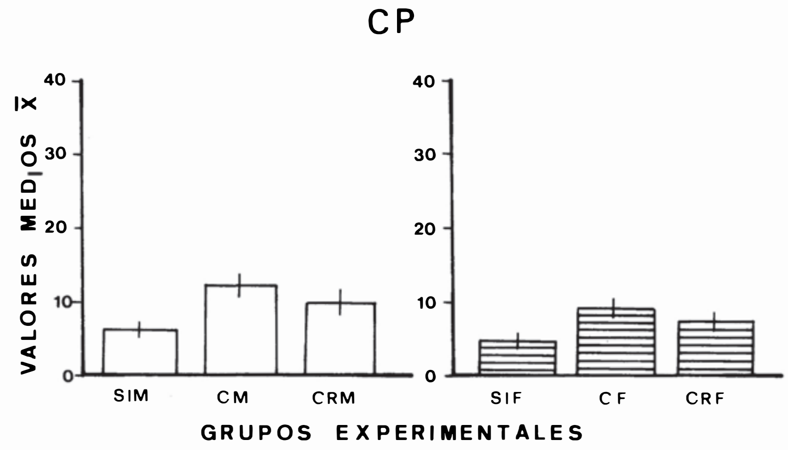

FIGURE 2. Copulatory postures (CP). Values are means±SE. SIM: socially isolated males; CM: control males; CRM: crowded males; SIF: socially isolated females; CF: control females; CRF: crowded females.

[Posturas copulatorias (CP) Los valores son medias ±EE). SIM: machos socialmente aislados; CM: machos controles; CRM: machos hacinados; SIF: hembras socialmente aisladas; CF: hembras controles; CRF: hembras hacinadas.]

Discussion

The present results indicate a negative social isolation effect on sexual performance of male and female rats , expressed as a significant decrease in their non copulatory postures (basically, pre-copulatory behaviours). These data support the view that social contact (stimulation) during development is necessary in the rat for a complete sexual behaviour display in the adulthood (Gruendel & Arnold, 1974; De Catanzaro & Gorzalca, 1979a; Chambers, 1982). The diminished ability animals reared in the social isolation to receive and interpret sexual cues, and to properly respond to this kind of stimuli, might account for their decreased precopulatory activity. In this respect, "social play" might be a major component of the necessary social contact during the rat's early development, although this point remains controversial (Gruendel & Arnold, 1974; Hole et al., 1986). Several data suggest some overexcitation or hyperreactivity in the isolates, which might prevent a well-integrated copulatory sequence (Hansen, 1977; Chambers, 1982). On the other hand, certain behaviours that can be considered as preliminary patterns of the copulatory activity *per se*, and not only the copulatory behaviour, appear to be dependent on male gonadal hormones (Hetta & Meyerson, 1978; Thor & Holloway, 1986). In this sense, a decreased androgen level has been found in socially deprived rats (Dessi-Fulgheri et al., 1976), which might be involved in the decreased precopulatory behaviour shown by male social isolates. It has been claimed that the impaired sexual behaviour found after social isolation might be due to a delay in the integration of copulatory processes rather than to a complete and irreversible disruption of the male's sexual performance (Chambers, 1982). Although no final conclusions can be drawn from the present results, as far as the copulatory behaviour of the isolated animals is concerned, a tendency to a decrease in copulatory postures was observed. As only a single short-duration test was carried out, we might expect that an improved copulatory activity would be shown by the isolates after repeated testing, suggested by previous reports (Chambers, 1982). The increased emergence times shown by the social isolated males might be interpreted as a delay in partner recognition, while the increased vocalization task of the isolated females might have been acting as an inhibitory stimulus for the approaching males. Previous data indicate that only when a complete isolation treatment is imposed (including a severe loss of sensory information), a significant impairment in the sexual behaviour of males is observed (Hole et al., 1986). The present results suggest that remarkable effects on sexual performance can be observed even when the isolation treatment does not involve such a severe loss of sensory inputs. However, these effects appear to be more complex and less drastic. In addition, it must be noted that the present work involved pairs of animals bred under the same social conditions. The crowding treatment given seems to be insufficient to cause marked effects in the animal's sexual behaviour. It is likely that the utilization of sexually receptive females may have masked some possible alterations in this case. As crowded females showed no clear modifications in their sexual behaviour during the test, crowding can be thought to affect certain mechanisms which act before and after this point, rather than specific sexual patterns. In fact, crowding might influence the females' ovarian cyclicity and the gestational processes more than their sexual performances *per se*. As noted above, a physiological approach to the effects of high population density is reported in most of the literature on this topic. In this sense, high social density appears to result, among other alterations, in decreased fertility and diminished growth and survival of the subsequent generations. The overall modifications have been considered as an endogenous mechanism that might be included in a wider process involved in the General Adaptation Syndrome, and that would serve to the social group homeostasis (Gray, 1971; Christian, 1979). On the other hand, the sexual maturation of female rats appears to be unaffected by social isolation (a normal development of the oestrous cycle is observed) which, in contrast, induces various changes in their sexual performance

(Hansen, 1977). Further research is needed to clarify the potential differential influence of these two extreme social conditions (isolation and crowding) on the physiological and behavioural factors involved in sexual processes. Moreover, a study on the influence of environmental "socialization" upon the sexual behaviour of previously socially isolated rats might provide interesting data about a potential reversal of the effects of social isolation upon sexual performance.

Resumen

Efectos del aislamiento social y del hacinamiento sobre el comportamiento sexual de la rata (Rattus norvegicus).

Diversos estudios han puesto de manifiesto la importancia de los factores de experiencia social durante el desarrollo, en cuanto a su influencia sobre la expresión del comportamiento sexual en edad adulta. En concreto, los efectos del aislamiento social sobre el comportamiento sexual en roedores parecen depender de la especie en estudio. En el caso de la rata, parece necesaria una experiencia social normal durante el desarrollo para el despliegue, en edad adulta, de un comportamiento sexual apropiado. Sin embargo, se requiere una mayor clarificación sobre el grado de severidad (privación de estímulos) del tratamiento de aislamiento que es capaz de producir un empeoramiento significativo de la actividad sexual de la rata de laboratorio. Por otro lado, se vienen utilizando, en este tipo de trabajos, hembras criadas en un ambiente social normal para pruebas de sexualidad con machos sometidos a aislamiento social. Se requiere una nueva aproximación que, mediante la observación de parejas de animales criados ambos en las mismas condiciones sociales, permita una adecuada valoración del activo papel de la hembra durante el desarrollo de las interacciones sexuales.

En el presente trabajo se aborda el estudio de los efectos del aislamiento social sobre el comportamiento sexual de la rata, mediante la utilización de parejas de animales criados en las mismas condiciones de alojamiento. Se pretende aportar información adicional sobre qué grado de severidad del tratamiento puede producir efectos notables sobre diferentes componentes del comportamiento sexual. Asímismo se estudian los efectos del hacinamiento sobre pautas sexuales concretas de la rata, ya que la información disponible se refiere predominantemente a factores fisiológicos, indicando efectos negativos sobre los mecanismos reproductores. También en este caso se utilizan parejas de animales criados ambos en condiciones de hacinamiento.

Se utilizaron 88 ratas albinas (*Rattus norvegicus*), machos y hembras de la cepa Sprague-Dawley. Los animales fueron alojados, a partir del destete (22 días de edad), según tres diferentes condiciones sociales: aislamiento social, condiciones estándar (5 animales del mismo sexo por jaula) y hacinamiento (15 animales del mismo sexo por jaula). Los animales alojados individualmente tenían acceso a diversos estímulos (excepto cutáneos) procedentes del resto de los animales que vivían en el mismo estabulario. Las pruebas de sexualidad (de diez minutos de duración) se realizaron con animales adultos sin experiencia sexual previa (un único "test", por pareja). Se utilizaron hembras en estro natural para evitar cualquier manipulación que pudiera enmascarar posibles alteraciones inducidas por los tratamientos. Se registraron pautas tanto de carácter no consumatorio (básicamente precopulatorias) (posturas no copulatorias, NCP), como de naturaleza típicamente consumatoria (posturas copulatorias, CP). Se midió también la tasa de vocalización de las hembras y el tiempo transcurrido desde el comienzo de la prueba hasta la aparición del primer contacto físico con señales sexuales (tiempo de emergencia, ET) en los machos.

Los resultados indican un efecto significativo del aislamiento social sobre el comportamiento sexual no consumatorio de los machos tratados ($p<0,005$), así como una disminución significativa de las posturas no copulatorias de las hembras

aisladas frente a las hacinadas ($p<0,05$) (fig.1). Los machos individuales mostraron también un aumento en los tiempos de emergencia ($p<0,05$) (tabla I). No se encontraron efectos significativos del hacinamiento sobre el comportamiento sexual de los animales tratados.

Los presentes resultados indican un efecto negativo del aislamiento social sobre el comportamiento sexual de ratas macho y hembra, que se expresa como una disminución significativa en sus comportamientos precopulatorios. Estos datos vienen a insistir en la importancia de la estimulación consocial durante el desarrollo del animal para que éste pueda, en época adulta, desarrollar un comportamiento sexual completo. La actividad precopulatoria disminuída de los aislados podría explicarse por una pérdida en su capacidad para recibir e interpretar las señales con significación sexual, así como para responder adecuadamente a este tipo de estímulos. Una cierta hiperreactividad o sobreexcitación, previamente observada en estos animales, podría también evitar una secuencia copulatoria bien integrada. Los tiempos de emergencia aumentados mostrados por los machos socialmente aislados podrían interpretarse como un retraso en el reconocimiento de la pareja. Datos previos indicaban que sólo un tratamiento de aislamiento completo (con pérdida severa de información sensorial) es capaz de producir un empeoramiento significativo del comportamiento sexual de los machos. Los presentes resultados sugieren que pueden observarse efectos importantes sobre dicho comportamiento incluso cuando el tratamiento de aislamiento no implica una pérdida tan severa de estimulación sensorial, al menos cuando se estudian parejas de animales criados ambos en aislamiento social.

El tratamiento de hacinamiento utilizado en este trabajo ha resultado insuficiente para producir unos efectos significativos sobre su comportamiento sexual. El hecho de haber elegido hembras sexualmente receptivas para las pruebas puede haber enmascarado, en este caso, posibles diferencias, hecho que por otra parte, podría indicar que las perturbaciones inducidas por el hacinamiento no se manifiestan en la conducta sexual de las hembras en estro frente a machos adultos, sino que seguramente afectan a mecanismos fisiológicos que actúan antes y después de ese momento, incidiendo sobre la periodicidad sexual de la hembra o sobre los procesos gestacionales. Por otra parte, datos previos indicaban que el aislamiento no afecta la maduración sexual de la rata hembra aunque sí modifica su comportamiento sexual.

Se requieren nuevos trabajos que contribuyan a un mayor esclarecimiento de la posible influencia diferencial de las dos condiciones extremas de población (aislamiento y hacinamiento) sobre los factores fisiológicos y comportamentales implicados en los procesos sexuales. Asímismo, sería interesante un próximo estudio sobre la influencia de la "socialización" ambiental en el comportamiento sexual de la rata, tras el período de aislamiento social, ésto es, la posible reversibilidad de los efectos de dicho tratamiento.

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