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The rat resident-intruder paradigm- a model for the Involuntary Subordinate Strategy (ISS)

The rat

Rats are members of the family Muridae which contains mice and moles (Barnett, 1975). Only a few of the 300 species of the genus Rattus are commensal with man. Laboratory rats are all derived from Rattus norvegicus. This is a burrowing rat which recently ousted the climbing Rattus rattus from temperate zones; sometimes the two species co-exist, norvegicus in the basement and rattus in the attic. Rats explore a strange environment and create paths marked with scent; this is thought to help them escape from cats. Wild rats commensal with man avoid new objects, or any change in a familiar environment; this "neophobia" is thought to represent selection for rats which avoid traps and bait.

Rats do not form hierarchies but male rats divide naturally into three status groups (Barnett, 1975, pp. 125-6):

> Alphas move about without hesitation or any attempt to take flight from other rats. They are the only rats that attack.
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> Secondly, omegas are the result of defeat by one or more alphas. Such rats flee at the approach of an alpha. In confined colonies omegas, after a day or two of persecution, are marked by their slow movements, drooping posture and bedraggled appearance. They lose weight, and die if not removed. A third category is needed for rats which, after defeat, adapt themselves to an inferior role: they have been called betas. They endure defeat and succeed in feeding with enough freedom to gain weight. Omegas and betas associate together without conflict: no "hierarchy" develops.
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> In unconfined colonies, adult males probably vary in status from alpha to beta; any rat with an omega status would soon die or emigrate. The observations of Telle on natural populations, though not detailed, conform with this.

Laboratory rats have largely lost the neophobia of wild rats, nor do they struggle or bite when handled. The males are less aggressive to other male rats. It is probable that the tameness to handling and the reduced intraspecific attack behaviour are mediated by different neural systems (Miczek, personal communication).

Male rats brought up together do not usually attack each other. Even strange rats introduced to the same cage at the same time do not fight. But if one male is in the cage for ten or more minutes before the introduction of a second rat, it may attack the intruder, and, unless the intruder is much bigger, the resident usually wins the fight and the two rats then have a dominance/subordination relationship. The process is complete in a few hours, after which there is little fighting; aggressive acts by the intruder rat fall to zero while aggressive acts by the dominant former resident take up less than 1% of behavioural time.

The defeated intruder rat can be studied while still in the cage of the resident, after variable lengths of time, or the intruder can be removed after the defeat experience and kept in isolation (or with other non-aggressive rats) and then studied after variable periods, and compared with the dominant rats and with rats not subjected to agonistic experience. In the first two sections to follow, the defeated rats were left in the presence of the winners until they were studied; in the last two sections, they were removed from the presence of the winners and studied under conditions of isolation for periods up to two months.

Four studies of defeated rats

Shortening of life span

The Blanchards and their colleagues at the University of Hawaii studied groups of rats composed of three males and three females over their total life span (Blanchard et al., 1993). The groups rapidly formed stable male hierarchies of one dominant rat and two subordinates who showed little mutual aggression. The subordinate males showed greatly reduced time on the surface (out of their burrows), and they showed vary maladaptive responses to the presence of a cat. After a cat had been placed briefly on the surface, the dominant rats increased their episodes of peeking out of the burrow, but each episode was greatly shortened; the subordinate rats did not increase the number of peeks, but each peek was greatly prolonged, which suggested to the experimenters that "the subordinate males were not obtaining or processing information very effectively".

They found increased plasma corticosteroids and reduced corticosteroid binding globulin in the plasma of subordinate male rats; some subordinate rats failed to give the normal increase in corticosteroid level to restraint stress, and these non-responders had lowered plasma testosterone. The subordinates in general had reduced plasma testosterone, their voluntary alcohol intake was raised, and their life-spans were shortened; they had increased 5-HIAA/5-HT ratios in a number of brain areas. Those subordinates who kept closer to the dominant males and slept in the same chambers lived longer than those subordinates who isolated themselves (cf the findings of von Holst in tree shrews). The subordinates who died early showed reduced aggressive and sexual behaviour 200 days before death, even below the reduced levels of those subordinate rats who survived the normal life span.

In other studies, both dominant and subordinate males showed adrenal and spleen enlargement, and thymus reduction, but only the subordinates showed reduction in testis weight.

In a study still to be reported in full, there was an increase in MRNA pro-opiomelanocortin in the pituitaries of subordinate rats.

Comment: In these studies the ISS is observed under semi-natural conditions. Subordination has a large effect on the general daily behaviour of the rat, even when agonistic behaviour is not occurring. The disordered behaviour of subordinates in response to a cat is interesting, suggesting an overlap between social and non-social defence systems. We do not yet know whether the altered 5-HIAA/5-HT ratios are picking up the same change detected in other ways by other investigators.

Raised plasma corticosteroid concentrations

Raab and his colleagues in Bordeaux studied both dominant and subordinate rats following ten days of cohabitation, and compared these with isolated controls and non-aggressive paired controls (Raab et al., 1986).

They classified the changes according to whether the changes in the two experimental groups (of dominant and subordinate rats) differed from the controls in the same or opposite directions.

Dichotomic changes occurred when the two groups diverged from controls in opposite directions. In the open field test, dominants explored the area during the whole test period whereas subordinates explored briefly and then withdrew to a corner and groomed themselves; controls had intermediate values. Dominants had higher prostate weights than controls, and subordinates lower prostate weights than controls.

Concomitant changes occurred when the two groups changed in the same direction compared to controls. Adrenal tyrosine hydroxylase was increased in both groups. This increase was presumably due to the fighting rather than to defeat or victory.

Specific changes occurred when only one experimental group differed from controls. The subordinate group lost about 5% of their body weight, and their plasma corticosteroid levels were approximately double those of all the other groups; their lymphocytes showed a reduced incorporation of radioactive thymidine when stimulated with various mitogenic agents.

Comment: These results suggest that there is an increase in adrenal medullary activity in both dominants and subordinates due to the agonistic interaction, and an increase in adrenocortical activity in subordinates only.

Insensitivity to morphine analgesia

Klaus Miczek and his colleagues at Tufts University in Massachusetts and the Scripps Research Institute in La Jolla have found changes lasting up to two months in morphine-induced analgesia of defeated rats (Miczek et al., 1982, 1990). This finding is based on work on stress-induced analgesia in rodents. When a mouse is defeated, its tail flick latency to heat stress is raised for several hours; that is, it takes longer to move its tail away from a hot object. The analgesia occurs in two phases. First, for ten minutes following defeat, there is a non-opioid analgesia, and this is thought to facilitate escape, so that reflex reactions to pain during escape do not interfere with the essential business of running away. Then, for about an hour, there is an opioid-mediated analgesia, which can be blocked with naloxone and other opioid antagonists; this is associated with bodily immobility, and is thought to be part of the "tonic immobility" response, in which the animal feigns death when in the jaws of a predator (Rodgers and Randall, 1987). Both these types of analgesia occur in rats in the presence of an aggressive resident: the non-opioid type occurs immediately the rat has signalled submission with "defensive upright", whereas the opioid analgesia occurs after the submitted animal has experienced a period of aggression from the winner.

Morphine also raises the flick latency, and during the few hours after defeat the response to morphine is enhanced in both mice and rats. Then the effect is reversed, and for a prolonged period, up to two months, the rat is relatively insensitive to the analgesic effect of morphine. The rats behave the same as rats fed with morphine, who rapidly develop tolerance. Both defeated mice and morphine tolerant mice show a morphine-withdrawal response to the morphine antagonist naloxone.

The authors suggest that defeat produces a prolonged activity of endogenous opiates in the area of brain subserving pain appreciation. Other actions of morphine are not affected by defeat; for instance, the rats continue to be able to discriminate injections containing morphine at the former level of accuracy (Miczek, 1991). Is this tolerance to morphine abolished by antidepressant drugs? We do not know.

Another effect of defeat is increased "emotionality" manifested by a reluctance to explore the open arm (rather than the enclosed arm) of a raised Plus-Maze (Heinrichs at al., 1992). This emotionality seems to be mediated by corticotrophin releasing factor in the central nucleus of the amygdala, because it can be blocked by the injection of small quantities of a CRF antagonist into this site but not into the dorsal striatum which served as a control. The effective dose for injection into the amygdala was 250 nanograms, both 125 and 500 ng being ineffective. Medium doses of CRF antagonist injected into the cerebral ventricles also abolished the defeat-induced emotionality. Abolition of the defeat-induced emotionality with the intra-amygdaloid CRF antagonist did not abolish the hypothalamo-pituitary-adrenocortical reaction to defeat (which is an activation prolonged more than the brief activation induced by fighting and/or winning). Nor did the abolition of the systemic cortisol response by an immune antagonist to CRF abolish the defeat-induced emotionality (Merlo Pich et al., 1993).

This work suggests that there are at least three independent long-term CNS reactions to defeat: an increase in endogenous opioid activity resulting in tolerance to the analgesic effects of morphine, CRF activity in the central nucleus of the amygdala causing "emotionality" manifested by cautiousness on an open maze, and the well-known hypothalamo-pituitary-adrenocortical response. Possibly one of these reactions is also responsible for the disorganisation of circadian rhythms which follows defeat (Tornatzky and Miczek, 1992).

Comment In the brains of these defeated rats and mice, we must be looking at the CNS component of the ISS. Therefore our depressive patients should show the same changes (allowing for species differences). If depressives are equivalent to endogenous morphine addicts, they should have an adverse reaction to morphine antagonists such as naloxone and naltrexone, but "the therapeutic effect of this drug on these diseases [schizophrenia, depression and tardive dyskinesia] was minimal or absent" (Nishikawa et al., 1994) and there was no mood change in alcoholics treated with 50 mg of naltrexone daily (Volpicelli et al, 1992). Nevertheless, the findings of the Miczek group indicate the need to study carefully the endogenous opiates in depression.

Since a CRF antagonist abolished defeat-induced emotionality, and had essentially the same effect as a benzodiazepine, we should look at the possibility of blocking amygdaloid CRF activity in depressed and anxious patients. The problem would be to cross the blood-brain barrier, hopefully without inducing a systemic corticoid deficiency. And would a CRF antagonist have the same disadvantages as the benzodiazepines, such as the development of tolerance? Perhaps benzodiazepines act by blocking central CRF, possibly indirectly.

Passivity reversed by antidepressant agents

Jaap Koolhaas and his colleagues at Groningen University in The Netherlands also used the rat resident-intruder paradigm, and they found that a lasting state of 'depression' followed a single defeat (Koolhaas et al., 1990). Their main measure of depression was the duration of immobility following sudden cessation of background noise. Normal rats have a brief period of immobility in this situation, but in the defeated rats the duration of the immobility was increased, and this effect lasted for several weeks. Interestingly, the change took two to three weeks to develop - one week after defeat there was negligible increase - at three weeks the time of immobility was double that of controls and the increased immobility was still present at ten weeks. The immobility was reduced to control levels by eight days treatment with clomipramine 5 mg per kg and this normalisation continued for at least three weeks after treatment was stopped. The increased immobility was also abolished temporarily by 12 hours sleep deprivation immediately before the test. The defeated rats also showed lengthened periods of immobility in a forced swimming test carried out ten weeks after defeat. They also lost weight for two weeks after defeat, but subsequently regained weight; and this shorter time scale suggests that there may be more than one separate process set in motion by the defeat experience. The increased immobility noticed by the Groningen group may be the same as the "reduced open arm of maze exploration" which the Tufts group called "emotionality"; although it is interesting that the Tufts group found the acoustic startle reflex to be unaffected by defeat, morphine and naloxone.

Comment: Passivity following defeat would not be surprising. What is interesting about these results is the time-scale. The passivity was not apparent a week after defeat, but increased gradually over a matter of two to four weeks and remained steady for a further six weeks. This is the sort of time scale which is seen in human depression following stressful life events. Also of interest is the abolition of the passivity by an anti-depressant drug, and its temporary suppression by sleep deprivation, which is known to have a similarly temporary effect on human depressive states. In later work the same authors found that a single defeat experience altered glutamate receptor binding in hippocampal CA3 area of male rats (Krugers et al., 1993).

Overall comment

All this work is very promising, and should be replicated on many other species. For me there are three main underlying questions:

1. To what extent are we dealing with a specific ISS and to what extent with a non-specific response to "stress"? Would the same changes follow the experience of being savaged by a cat? We know the response to a powerful con-specific is different to that to a powerful predator: for instance the rat does not show the submissive responses of "defensive upright" and "crouch" in response to a predator; is there an equivalent difference in CNS responses?

And what about the stress of "inescapable shock"? Into which functional module does the rat classify this experience?

We should not forget Keith Dixon's exciting, and, I think, still unpublished finding that amitriptyline blocks the hormonal response to social stress but not to non-social stress in rats. This suggests a differentiation of the social and non-social defence systems.

2. Is the rat ISS likely to be homologous to the human ISS? We know that useful things tend to evolve many times over, sometimes using very similar mechanisms but sometimes totally different ones. I have always been wary of rodents because it seems to me that in the rodent lineage the phasic switch which operates the ISS has been used up in dealing with climatic stress, and controls hibernation in those rodents that hibernate. In non-hibernating rodents it is probably inactive. This is just a hunch. It would be really useful to see the rodent work replicated in sugar gliders or better still in primates.

3. Tonic immobility is a predator avoidance strategy which does not occur in primates. It is thought that responses to intraspecific aggression evolved out of predator avoidance behaviour (Rodgers and Randall, 1987). Is it possible that primates have adapted the neural substrate of the tonic immobility reaction to create the ISS, and thus fashioned one of the strategies which have made group living possible?

Individual variations in defeat behavior

The Groningen workers noted that the development of a severe defeat reaction depended on the intensity of the conflict leading to defeat:

> Defeat of an aggressive, i.e. potentially dominant, male rat results in the development of long-term behavioral depression, whereas non-aggressive rats, i.e. potential subordinates, fail to develop such syndrome (Bohus et al., 1993).

This may be the rat equivalent of the difference between active and passive submission. If you submit actively, possibly having what John Birtchnell calls a need for lowerness, you do not need to get depressed; only if you are coerced into submission does the submission become passive and the features of the ISS appear.

There are two clear rat phenotypic responses to defeat, in one of which the defeated rat seeks the company of the winner and adopts a submissive role, and in the other it tries to get as far away as possible, and if prevented from doing this, it goes into a decline and dies. These two defeat behaviour patterns are identical to those described in the tree shrew by von Holst. We do not know whether these two "yielding strategies" are genetically determined, or whether they reflect social experience, or whether they reflect the severity of the defeat which induced the yielding.

It would not be surprising if, in mammals as a whole, there were two fundamental defeat strategies. One would aim for escape, and the search for a new territory; and we would expect this to be commoner in "territorial" species such as the tree shrew. If escape is blocked, which presumably would seldom occur under natural conditions, these animals suffer severe reactions, their physiology becomes disordered, and they die. Keith Dixon has called the condition of these animals "Arrested Flight" (Dixon et al., 1989). If their ISS is qualitatively the same but just more intense than animals allowed to escape, then they are good experimental subjects for studying the physiology of the ISS.

The other basic defeat strategy is based on what Michael Chance has called "reverted escape". The pulls of the group are greater than its pushes, so when defeated the animal seeks the company of the winner and adopts a subordinate role. If escape is impossible, this is clearly a more advantageous strategy. It is this strategy which makes group living possible, and we would expect it to be commoner in group living species such as Rattus norvegicus.

A third strategy has been observed in laboratory rats, and is presumably an artefact of inbreeding:

> Laboratory rats put in a strange cage with a resident wild male may not evoke typical attack; but, if they do, they tend not to respond at all: instead of showing the signs of disturbance observed in wild rats, they may continue their exploration of the cage. The same behaviour has been observed in rats of the second generation derived from a cross between wild and laboratory rats. It is a remarkable sight to see a laboratory or hybrid male moving slowly around a cage while a resident wild male vigorously postures or leaps at it without producing any evident alteration of behaviour. (Barnett, 1975, p. 127).

We do not know whether this "coolness" on the part of the laboratory rat protects it from the various changes of the ISS. If so, the laboratory rat seems to have evolved the genetic basis required for a truly hedonic culture.

Individual variation in attack behaviour

There is a lot of genetic variation in attack behaviour; in many strains, there is a proportion of "attackers" and a proportion who do not attack under any circumstances - even before the rats have been given the opportunity to differentiate into alphas, betas and omegas. Miczek et al. (1993) state that only 60-70% of male Long-Evans residents fight with an intruder. Barnet (1975) states: "If a strange male approaches a non-aggressive male on the latter's territory, the resident may crawl under the stranger instead of attacking" (p. 107). Attack behaviour may be altered by drugs; for instance, small doses of alcohol or benzodiazepines enhance attack behaviour in mice, not by reducing latency to attack, but by increasing the duration of each bout of fighting (Miczek at al., 1993).

There is room for a lot of research into these matters, both in the Muridae and in other families.

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References

Barnett SA (1975) The Rat: a Study in Behaviour. Chicago: University of Chicago Press.

Blanchard RJ, Yudko EB, Rodgers RJ & Blanchard DC (1993) Defence system psychopharmacology: an ethological approach to the pharmacology of fear and anxiety. Behavioral Brain Research, 58, 155-165.

Blanchard DC, Sakai RR, McEwen B, Weiss SM & Blanchard RJ (1993) Subordination stress: behavioral, brain and neuroendocrine correlates. Behavioral Brain Research, 58, 113-121.

Dixon AK, Fisch HU, Huber C & Walser A (1989) Ethological studies in animals and man: their use in psychiatry. Pharmacopsychiatry, Supplement 1, volume 2, 44-50.

Heinrichs SC, Merlo Pich E, Miczek KA, Britton KT & Koob GF (1992) Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. Brain Research, 581, 190-197.

Holst, D. von (1986) Vegetative and somatic components of tree shrews' behavior. Journal of the Autonomic Nervous System, Suppl., 657-670.

Koolhaas, J.M., Hermann, P.M., Kemperman, C., Bohus, B., Hoofdakker, R.H. & Beersma, D.G.M. (1990) Single social defeat in male rats induces a gradual, but long lasting behavioural change: a model of depression? Neurosci. Res. Comm., 6, 35-41.

Krugers HJ, Koolhaas JM, Bohus B & Korf M (1993) A single social stress experience alters glutamate receptor binding in rat hippocampal CA3 area. Neuroscience Letters, 154, 73-77.

Merlo Pich E, Heinrichs SC, Rivier C, Miczek KA, Fisher DA & Koob GF (1993) Blockade of pituitary-adrenal axis activation induced by peripheral immunoneutralisation of corticotropin-releasing factor does not affect the behavioral response to social defeat stress in rats. Psychoneuroendocrinology, 18, 495-507.

Miczek KA, Weerts EM & DeBold JF (1993) Alcohol, benzodiazepine-GABA receptor complex and aggression: ethological analysis of individual differences in rodents and primates. Journal of Studies in Alcohol, Supplement 11, 170-179.

Miczek KA (1991) Tolerance to the analgesic, but not stimulus discriminative stimulus effects of morphine after brief social defeat in rats. Psychopharmacology, 104, 181-186.

Miczek KA, Thompson ML & Tornatzky W (1990) Subordinate animals: behavioral and physiological adaptations and opioid tolerance. In: Stress: Neurobiology and Neuroendocrinology ed Brown MR, Koob GF & Rivier C. New York: Marcel Dekker, Pp. 323-357.

Nishikawa T et al. (1994) Decreased polydipsia in schizphrenic patients treated with naloxone. American Journal of Psychiatry, 151,

947.
Raab A, Dantzer R, Michaud B, Mormede P, Taghzouti K, Simon H & Le Moal M (1986) Behavioral, physiological and immunological consequences of social status and aggression in chronically coexisting resident-intruder dyads of male rats. Physiology and Behaviour, 36, 223-228.

Rodgers RJ (1995) Neuropharmacological aspects of adaptive pain inhibition in murine "victims" of aggression. Aggressive Behavior, 20, in press.

Rodgers RJ & Randall JI (1987) On the mechanisms and adaptive significance of intrinsic analgesia systems. Reviews in the Neurosciences, 1, 185-200.

Schuurman, T. (1980) Hormonal correlates of agonistic behavior in adult male rats. Progress in Brain Research, 53, 415-420.

Tornatzky W & Miczek KA (1993) Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. Physiology and Behavior, 53, 983-993.

van de Poll NE, Smeets J, van Oyen HG & vaan der Zwan SM (1982) Behavioral consequences of agonistic experience in rats: sex differences and the effects of testosterone. Journal of Comparative and Physiological Psychology, 96, 893-903.

Volpicelli JR, Alterman A, Hayashida M & O'Brien CP (1992) Naltrexone in the treatment of alcohol dependence. Archives of General Psychiatry, 49, 876-880.