Single exposure to stressors causes long-lasting, stress-dependent reduction of food intake in rats

Astrid Valles, Octavi Martí, Arantxa García, and Antonio Armario

Departament de Biologia Cel·lular, Fisiologia i Immunologia, Unitat de Fisiologia Animal, Facultat de Ciències, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

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Exposure to some physical stressors, such as surgery, inflammation, or endotoxins, triggers a number of physiological processes that persist far beyond the initial challenge. In these cases, a relatively long-lasting (days) effect of such stressors on behavioral and physiological variables, including food intake, might be expected (22). However, in recent years, attention has been paid to long-lasting effects of a single exposure to predominantly psychological stressors (1, 3, 19, 31, 36, 42). The only acute psychological stressor reported to cause changes in food intake persisting for several days is social defeat (26, 34). The extent to which long-lasting changes in food intake caused by social defeat can be extended to other psychological stressors is not known. However, from a theoretical point of view, it is important to distinguish between two alternative hypotheses regarding stress-induced anorexia: 1) it might reflect qualitative, phylogenetically developed aspects of particular stressors; or 2) it might be merely related to quantitative aspects of stressors (i.e., intensity and duration) independently of their nature.

Long-lasting reduction in food intake caused by social defeat, which might be an ethologically relevant animal model of depression (19), would be a very specific property of this particular stressor and a reflection of a general decrease in all motivated behaviors allowing subordinate animals to avoid dominant conspecifics and survive. Alternatively, other severe and predominantly psychological stressors might share with social defeat long-lasting effects on food intake, suggesting a...
MATERIAL AND METHODS

Animals. Adult male Sprague-Dawley rats were obtained from the breeding center of the Universitat Autònoma de Barcelona. The animals were 45–55 days old on arrival at the laboratory. They were housed one or two per cage, depending on the experiment, for ≥1 wk before starting the experiments, under a standard 12:12-h light-dark cycle (lights on from 7 AM to 7 PM) and at 22 ± 1°C. Food and water were given ad libitum. Food intake and body weight gain were periodically controlled, always at the same time of the day and just before the stress session on the day when stress was applied (from 8 to 9 AM). All the experimental protocols were approved by the Committee of Ethics of the Universitat Autònoma de Barcelona.

Experiment 1. The animals, housed two per cage, were assigned to three groups: rats injected intraperitoneally with isotonic saline (IS; group 1), rats injected intraperitoneally with lipopolysaccharide (LPS; group 2), and rats injected intraperitoneally with IS and subjected to Imo for 2 h (group 3). LPS (Escherichia coli, 055:B5; Sigma Chemical) was dissolved in sterile water, further diluted in sterile saline, and injected in a volume of 2 ml/kg at a dose of 1 mg/kg. To immobilize the rats, their four limbs were taped to metal mounts attached to a wooden board, and their head movements were restricted with two metal loops around the neck (20). Food intake and body weight were controlled until poststress day 3, when the animals were used for other experimental purposes.

Experiment 2. Because we previously demonstrated that individual housing did not interfere with the effects of stress on food intake and body weight gain (10), in experiment 2 rats were housed one per cage to reduce the number of animals. The animals were assigned to three groups: rats injected with IS (2 ml/kg ip; group 1), rats injected with LPS (1 mg/kg ip; group 2), and rats injected with IS and subjected to Imo for 2 h (group 3). Because experiments with LPS quite often require temperature measurements and we wanted to know the influence of mild stressful procedures on food intake, a rectal probe was used to measure rectal temperature at 30-min intervals for 4 h from the beginning of stress.

Experiment 3. To study the effect of changing the period of exposure to Imo on food intake, 32 rats, housed 2 per cage, were assigned to 4 groups: controls (group 1), rats subjected to 20 min of Imo (group 2), rats subjected to 2 h of Imo (group 3), and rats subjected to 6 h of Imo (group 4). Food intake was controlled over 5 days after stress.

Experiment 4. Experiment 4 was designed to study the effect of various doses of LPS on food intake over 5 days after stress. Individually housed animals (7–8/group) were assigned to IS or LPS groups receiving one of the following LPS doses: 50, 250, and 1,000 µg/kg. To demonstrate a dose response with another independent variable, rectal temperature was measured with a thermistor probe just before IS or LPS four times at 45-min intervals, and finally at 5 h 45 min after the injection.

Statistics. Data were analyzed by two-way multivariate ANOVA (MANOVA), with repeated measures for the factor time (days for food intake and body weight gain and hours for rectal temperature). When a significant interaction between the two main factors was found, the different groups were compared at each time point with one-way ANOVA followed by post hoc comparisons with the Student-Newman-Keuls (SNK, P < 0.05) or the paired t-test.

RESULTS

Experiment 1. Figure 1 shows food intake of the animals during the days after the initial treatments. Two-way MANOVA for repeated measures revealed significant effects of treatments, time (days), and treatment × time interaction on food intake (P < 0.001 in all cases). On poststress day 1, there was a marked decrease of food intake in the groups that received LPS or were subjected to Imo, the effect being more marked in the LPS than in the Imo group (SNK). On poststress days 2 and 3, although food intake remained lower in these two groups than in controls, food intake of the Imo group became lower than that of the LPS group. As shown in Fig. 1, there were significant effects of treatments, time, and treatment × time interaction on body weight gain (2-way MANOVA, P < 0.001 in all cases). There was a significant decrease of body weight on the
day after LPS injection or Imo, but thereafter the body weight gain in the LPS group was parallel to that of controls, whereas the Imo group did not gain weight.

Experiment 2. The two-way MANOVA for repeated measures revealed significant effects of treatments ($P < 0.02$), time ($P < 0.001$), and treatment $\times$ time interaction ($P < 0.001$) on food intake (Fig. 2). Control rats showed a transient decrease of food intake on day 1 after saline injection and temperature probe application compared with prestress levels of food intake ($P < 0.03$, paired $t$-test). However, in the LPS and Imo groups, there was a marked decrease in food intake on the day after stress compared with the control group, the effect being greater after LPS (SNK). On poststress day 2 to poststress day 4, all Imo groups showed a significant reduction of food intake compared with controls, with no differences among the three Imo groups. On poststress day 5 the effect of Imo did not reach statistical significance.

Experiment 3. The MANOVA revealed significant effect of treatments, days, and treatment $\times$ days interaction ($P < 0.001$ in all cases). Post hoc analysis revealed that on poststress day 1 all Imo groups showed a significant reduction in food intake compared with controls (Fig. 3), with the effect of 6 h of Imo greater than the effect of 20 min or 2 h of Imo (SNK). From poststress day 2 to poststress day 4, all Imo groups showed a significant reduction of food intake compared with controls, with no differences among the three Imo groups. On poststress day 5 the effect of Imo did not reach statistical significance.

Fig. 2. Effects of LPS administration and 2 h of Imo stress on food intake and body weight gain. All groups received saline, and rectal temperature was measured repeatedly on the day of stress. Values are means $\pm$ SE (n = 5–6 for body weight and food intake). *Significantly different from control (saline) and LPS groups within the same poststress day; $\dagger$significantly different from control group within the same poststress day (Student-Newman-Keuls test, $P < 0.05$). $\ddagger$Significant difference between day 1 and day 0 in the saline group (paired $t$-test).

Fig. 3. Effects of different periods of exposure to Imo on food intake. Values are means $\pm$ SE (n = 4/group). $*\ddagger$Significantly different from all Imo groups within the same poststress day; $*\ddagger$significantly different from 20-min and 2-h Imo groups within the same poststress day (Student-Newman-Keuls test, $P < 0.05$).
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a reduction of food intake, the effect being marginally significant ($P = 0.07$) on poststress day 3.

**DISCUSSION**

The present results indicate that exposure of rats to physical (LPS) and psychological (Imo) stressors caused changes in food intake that persisted for some days after initial exposure to the stressors. A severe psychological stressor such as Imo caused a longer-lasting anorexia than a physical stressor such as LPS administration, despite an initially greater degree of anorexia caused by LPS in the first 24 h after the stressor, suggesting that, in comparing different stressors, the initial anorexia is not predictive of its further effects.

In experiment 1 the effect of 2 h of exposure to Imo on food intake was compared with the effect of LPS administration (1,000 µg/kg). The two conditions are characterized, in our hands or on the basis of literature data, as inducing anorexia and activation of the pituitary-adrenal axis (6, 40; unpublished data). To allow appropriate comparisons between groups, control and Imo animals received IS intraperitoneally. LPS and Imo caused profound reductions in food intake compared with controls, with the effect remaining significant 3 days later. Whereas the anorectic effect of LPS was greater than that of Imo in the 24 h after exposure to the stressors (day 1), the opposite pattern was found on poststress days 2 and 3. Body weight gain was a reflection of the changes in food intake; body weight gain of LPS-treated rats roughly paralleled that of control animals after poststress day 1, whereas Imo rats did not gain weight. In an additional experiment the anesthetic cocktail Equithesin also caused a more marked anorexia than Imo on day 1, but on day 3 the effect of Equithesin was no longer observed, whereas that of Imo was (unpublished data). From the above results, it is clear that physical and psychological stressors are able to cause profound changes in food intake, the intensity of the effects and its duration being dependent on the particular stressful situation.

The anorectic effects of Imo were more persistent than those of LPS administration or Equithesin anesthesia, despite a greater degree of anorexia caused by both treatments in the first 24 h. These data suggest that initial anorexia is not predictive of the protracted impact of a stressor on food intake and that long-lasting effects of a severe psychological stressor might be greater than the effects of physical stressors. To better know the dynamics of recovery of normal food intake after stress, in experiment 2 we compared the response to LPS (1,000 µg/kg) with the response to Imo (2 h). Because temperature is an important variable to be measured in response to LPS and other stressors, we also evaluated the impact of repeated rectal temperature measurement on food intake. This procedure per se reduced food intake only on poststress day 1 and to a much lesser extent than LPS or Imo. A greater degree of anorexia was observed as a result of LPS administration than as a result of Imo on the day after stress, but a faster recovery was observed in LPS than in Imo rats. As a result, normal food intake was observed on day 4 and thereafter in LPS-treated rats, whereas in Imo rats, normalization was only observed on poststress day 8.

Although we previously reported (25) that the degree of anorexia caused by chronic (7 days) exposure to Imo was not dependent on the duration of daily exposure to the stressor (15 min, 1 h, or 4 h), the possibility remains that the duration of a single exposure to Imo could be positively related to the duration of anorexia. In experiment 3, rats were exposed to IMO for 20 min, 2 h, and 6 h, and we found that the only significant difference between the Imo groups was a greater anorexia of the 6-h Imo group than in the other two groups on poststress day 1. Thereafter, the three groups showed a similar pattern of food intake. With briefer exposures to Imo (1 min), only a slight reduction in food intake, lower and of shorter duration than that caused by 20 min of Imo, was observed (unpublished data), suggesting that duration of exposure to the stressor is also influencing, within certain limits, initial and delayed anorexia. However, the impact of
stressors on food intake is clearly more dependent on their nature than on their duration.

To further study the relationship between the intensity of a stressful situation and anorexia, various doses of LPS were administered. The effect of LPS administration on rectal temperature was dose dependent and followed the expected pattern described in the literature under similar conditions (9, 11, 33), the two higher doses of LPS causing hypothermia. The reduction of food intake observed in the 24 h after LPS administration was monotonically dependent on the doses of LPS. In addition, the greater the anorexia observed on post-stress day 1, the longer the period of recovery of normal food intake lasted. These data indicate that LPS-induced anorexia was dose dependent and that duration of LPS-induced anorexia was positively related to the intensity of anorexia observed during the first 24 h, unlike that found when Imo and LPS administration were compared.

Because stressors such as LPS and Equithesin caused a marked anorexia on the day after administration but recovery was more rapid than with Imo, the prolonged negative effects of exposure to some stressful situations on food intake reveal an important characteristic of severe psychological stressors vs. physical stressors that was not shown during the initial response. This suggests multiple physiological mechanisms underlying stress-induced anorexia. There is consistent evidence in the rat that corticotropin-releasing hormone (CRH) might be involved in anorexia caused by restraint (37, 38) and interleukin-1 (41). More recently, it has been demonstrated, using a specific nonpeptide CRH antagonist, that CRH type I receptor might be involved in emotional stress-induced anorexia (13). However, the stress caused by the simulation of adrenalectomy has been observed to cause anorexia in CRH-deficient transgenic mice (15), suggesting that other factors are also involved with this particular stressor in mice. There is also evidence that even the response to different immune challenges might involve different mechanisms (21). Finally, the biological meaning of the finding that α-melanocyte-stimulating hormone blocks a wide range of foot shock-induced changes, including anorexia (27), is questioned by the negative evidence that the arcuate proopiomelanocortin system is activated under stress (23).

Clearly, more studies are needed to know the neurobiological substrate of stress-induced changes in food intake, but the present findings suggest that stressor-specific physiological mechanisms may be involved in stress-induced anorexia.

The present results clearly demonstrate that long-lasting effects of stress on food intake are not restricted to social defeat (26, 34) but might be extended to other types of psychological stressors. The finding that some experimental manipulations previously assumed to induce only transient physiological alterations actually caused such long-lasting effects on food intake was somewhat unexpected and is in contrast with the results reported by Rybkin et al. (35), who observed that 3 h of exposure to restraint only reduced food intake in the 24 h after the stressor, but not thereafter. However, the pituitary-adrenal response to restraint in tubes is far lower than that to Imo (5, 7), so we can assume that the intensity of the stressful situation could markedly influence the speed of recovery of normal food intake. In addition, these long-lasting effects of stress on food intake, particularly prolonged in the case of a severe, mainly psychological stressor such as Imo (32), are compatible with long-lasting changes in the reactivity to novel environments and other stressful situations caused in rats by social defeat (19), cat exposure (1), and foot shock (42).

It is difficult to speculate about the biological meaning of anorexia caused by psychological stressors. Stress-induced anorexia appears to be independent of hedonic properties of food (12, 14, 16) and reflects a general decrease in motivation to eat, since exposure to shock (2) or to restraint plus water immersion (44) has been found to reduce lever pressing for food in hungry rats. It is possible that exposure to traumatic situations could induce in those animals surviving the situation a reduction of food intake as well as other rewarded behaviors (i.e., exploratory activity, social activity, sexual drive) to reduce the possibility that they would again encounter the stressor. Although Imo, like foot shock, is unlikely to be a natural stressor for the rat, it might be a model for traumatic events in animals and humans (i.e., war, aggression, natural disasters) and share some neurobiological mechanisms with ethologically relevant stressors such as social defeat. Therefore, Imo could be used as a model to characterize the neurobiological consequences of traumatic events and the neurobiological mechanisms underlying such consequences.

The present results demonstrate that a single exposure to physical and psychological stressors caused anorexia, the intensity and dynamics of recovery of which are greatly dependent on the nature of the stressor. Whereas the effect of a particular physical stressor (LPS administration) was dose dependent, the effect of a severe psychological stressor such as Imo was only modestly dependent on its duration. Duration of stress-induced anorexia appears to reflect a qualitative rather than quantitative aspect of stressors and be a relevant characteristic of severe psychological stressors.

Perspectives

Exposure to a wide range of physical and psychological stressors results in partial anorexia. However, the particular characteristics of the stressors determining the degree of anorexia have been poorly characterized. In addition, with the exception of social defeat, the protracted effects of a single exposure to psychological stressors on food intake have not been studied. The present work addressed these questions and showed that the reduction of food intake caused by a single exposure to physical (LPS administration) and psychological (Imo) stressors persisted beyond the first 24-h period. Interestingly, the anorexia observed during the
initial 24-h period was not predictive of long-lasting anorexia, in that LPS showed a greater effect than Imo during the first 24 h but recovered faster. Apparently, a single exposure to a severe psychological stressor is able to cause a long-lasting anorexia, the biological meaning of which is unclear. Our results support the assumption that neurobiological mechanisms involved in stress-induced anorexia might be dependent on the particular stressor used and, within this context, Imo could be used as an animal model to study neurobiological consequences of exposure to traumatic events.

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