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# Apomorphine-susceptible and apomorphine-unsusceptible Wistar rats differ in their recovery from stress-induced ulcers

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**Abstract**

The aim of the present study was to investigate the effects of restraint-in-water-stress on gastric ulcerations in two fundamentally different types of animals: the apomorphine-susceptible (APO-SUS) and apomorphine-unsusceptible (APO-UNSUS) rats. APO-SUS and APO-UNSUS do not only differ in their susceptibility to the dopamine agonist apomorphine, but also in stress-induced release of mesolimbic dopamine and corticosterone. All three factors are known to either predict or be involved in gastric ulceration. The results showed that immediately after the stressor the ulcerations in APO-SUS and APO-UNSUS rats were not line-specific. On the contrary, the recovery from gastric ulceration varied between both types of rat: APO-SUS rats did not show any sign of recovery after 6 hours whereas APO-UNSUS rats significantly recovered during the period of 0–6 hr after the stressor. It is hypothesised that this difference is due to the fact that APO-UNSUS rats are characterised by a less and shorter-lasting stress-induced increase of corticosterone. This study provides evidence that the pathological effects of exposure to stressors significantly differ between APO-SUS and APO-UNSUS rats and that genetic factors may direct the process of recovering from ulcers.


*Keywords:* Dopamine; Corticosterone; Gastric; Ulcer; Selection line; Restraint-in-water-stress

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## Introduction

Gastric ulceration has long been viewed as the prototypic disease of stress [1] and, indeed, a great variety of stressors have been demonstrated to produce ulcers. These stressors may be more psychological, such as early social experience [2,3] or more physiological, such as alcohol [4].

A remarkable - but not totally unexpected - fact is that in humans as well as in animal models some individuals develop ulcers whereas others do not, notwithstanding the fact that the stressors they were exposed to were similar. Apparently large individual differences in vulnerability to stress-induced gastric ulcerations exist. In the present study we focus on this individual variation and investigate the effects of restraint-in-water-stress, one of the most common stress procedures in experimental ulcer research [5–8], on gastric ulcerations in two fundamentally different types of animals: the apomorphine-susceptible (APO-SUS) and apomorphine-unsusceptible (APO-UNSUS) rats. The APO-SUS and APO-UNSUS lines are the result of bi-directional selection for the dopamine agonist apomorphine. Unlike some mouse models they have not been genetically engineered and they represent the extremes of a normal, unselected population of Wistar rats [9–11]. They also vary for other neurobehavioural characteristics. Their response to stress, for instance, is essentially different. APO-SUS animals show a higher release of mesolimbic dopamine [10] and corticosterone [12] than APO-UNSUS animals. Both dopamine and corticosteroids are important factors in gastric ulcerogenesis. Dopamine plays a predominantly protective role in this process [5,13–17] whereas higher levels of corticosterone are associated with increased gastric ulceration [18–21]. Corticosterone also slows down the recovery process [22,23].

Given the significance of dopamine and corticosteroids in ulcerogenesis and the fundamentally different stress response in our animal model, we expect APO-SUS and APO-UNSUS animals are characterised by a line specific degree of gastric ulceration after restraint-in-water-stress. We anticipate that each of these lines has its own line-specific pattern of recovery from the stressor.

## Materials and methods

### *Subjects*

Subjects were male rats of the 26th and 27th generation of selection. In total, 31 APO-SUS and 38 APO-UNSUS were used. Both lines were bred and reared in the Central Animal Laboratory of the University of Nijmegen, the Netherlands. For a detailed description of the selection procedure, the reader is referred to [9]. Animals were housed in temperature-controlled rooms (21 $\pm$ 1.7 °C), with a fixed 12:12 light/dark cycle (lights on 0700). Litters were culled to 4 males and 4 females within 24 hours after birth. After weaning at postnatal day 28 the animals were housed with same sex littermates with a maximum of 3 animals per cage (42 $\times$ 26 $\times$ 15 cm.). Standard lab chow (RMH-B, Hope farms) and water were available *ad libitum* unless otherwise indicated. The animals were individually housed 46–60 days after birth. The restraint-in-water-stress experiments were carried out at the age of 70–82 days.

*Restraint device*

Animals were restrained in a transparent plastic cylindrical tube (height: 17.5 cm; diameter: 6 cm). Both the plastic bottom and the removable plastic cap of the tube contained 4 holes (diameter: 12 mm) while the tube itself also contained holes (n = 12; diameter: 8 mm) across the surface.

*Experimental procedure*

In total four experimental groups were created per genotype. In order to follow the effects of restraint-in-water-stress (RIWS) on gastric ulceration in time, three groups were formed: [i] a 0 hour group, in which the animals were immediately sacrificed after the RIWS (8 APO-SUS and 9 APO-UNSUS males), [ii] a 4 hour group, in which the animals were sacrificed 4 hours after the RIWS (9 APO-SUS and 11 APO-UNSUS) and [iii] a 6 hour group, in which the animals were sacrificed 6 hours after the RIWS (6 APO-SUS and 8 APO-UNSUS). A control group which was left undisturbed (no RIWS) was also included in the experimental design (8 APO-SUS and 10 APO-UNSUS).

Sixteen hours prior to the RIWS, animals were food deprived. Thirty minutes before the actual RIWS animals were moved to the experimental room. Rats were individually placed into the restraint tubes and immersed in the water tank (21 ± 1 °C) for 75 min. The upper part of the tube was left 5 cm above the water surface. Immediately after the RIWS, rats from the 4 h and 6 h groups were removed from the tube, dried and placed in their home cage. Animals from the 0 hour group were sacrificed immediately after the RIWS, control animals after the 30 min acclimatisation period. Animals were killed with an overdose of pentobarbital (narcovet®: 80 mg/kg).

*Stomach examination*

In order to determine the degree of gastric ulceration, stomachs were cut along the lesser curvature, everted, washed and swabbed clean. Next, each stomach was pinned on a wax table, so that the inner side became visible. Gastric ulcerations were defined as grey or reddish indurated and elongated strips embedded in healthy, pinkish mucosa. All strips were either completely reddish or just reddish in the inner part and surrounded by a grey outer part [24,25]. In this context, it has to be mentioned that stress-ulcers in rats are different from those in humans in that they are most commonly gastric instead of duodenal and they are generally transient [26]. They are characteristic of the premorbid stage of human ulcers [24,27].

Ulcerations were measured as either cumulative length or total number of spots. Measurements were performed blindly by four observers (SD, MvdE, EG, MH).

*Statistics*

As the reactivity of both the central dopaminergic system and the HPA-axis fundamentally differ between APO-SUS and APO-UNSUS, RIWS data were analysed per line using a 1-way ANOVA followed by a Student-Newman-Keuls post hoc analysis where appropriate.

Control groups were compared using the Student one sample *t* test. The inter-observer reliability was analysed by means of the non-parametric Spearman's correlation test, the correlation between the two measures by the parametric Pearson's test. Significant *P* values were set at 0.05.

## Results

### General

The inter-observer reliability was both very high and highly significant (length: $\rho = 0.98$; number: $\rho = 0.98$). Also, both measures, namely length and number, correlated so strongly ($r = 0.823$; $p \leq 0.001$) that only the lengths of ulcerations were analysed.

### Control experiments

Neither APO-SUS nor APO-UNSUS showed any sign of ulcerations [APO-SUS: $t(7) = 1.00$, $p = 0.35$; APO-UNSUS: $t(9) = 1.40$, $p = 0.20$].

### Restraint-in-water stress induced gastric ulcerations

RIWS resulted in considerable ulcerations in all APO-SUS and APO-UNSUS animals.

#### APO-SUS

Data are displayed in Fig. 1. All RIWS groups significantly differed from the control group ($p < 0.05$). No significant recovery was observed during the post-RIWS period of 4–6 hr as illustrated by the fact that neither the 4 hr group nor the 6 hr group significantly differed from the 0 hr group.

#### APO-UNSUS

Data are displayed in Fig. 2. All animals developed ulcerations after exposure to RIWS. Similar to APO-SUS animals, all RIWS groups significantly differed from the control group ($p < 0.05$). By contrast, APO-UNSUS animals did recover from the RIWS in comparison to those animals immediately sacrificed, but only after 6 hours ($p < 0.05$). Given the fundamental differences between APO-UNSUS and APO-SUS rats, the data were not analysed across the lines; nevertheless, comparison of the data

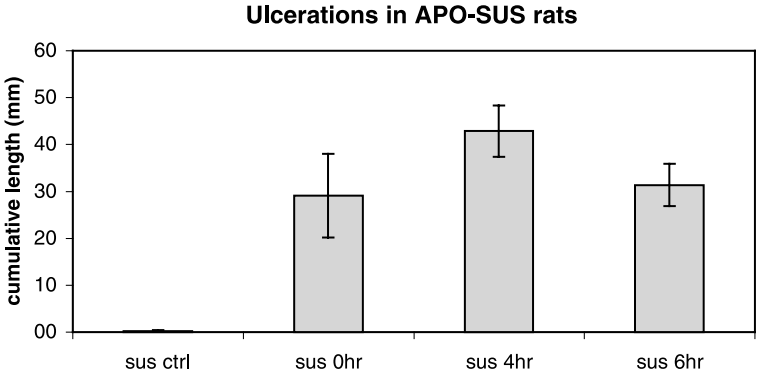


Fig. 1. Ulcerations (mm) in APO-SUS rats after 75 minutes of restraint-in-water stress (RIWS) (Mean ± SEM). RIWS produced ulcerations in all animals. No recovery was observed in the post-RIWS period, neither 4 nor 6 hours after the stressor.

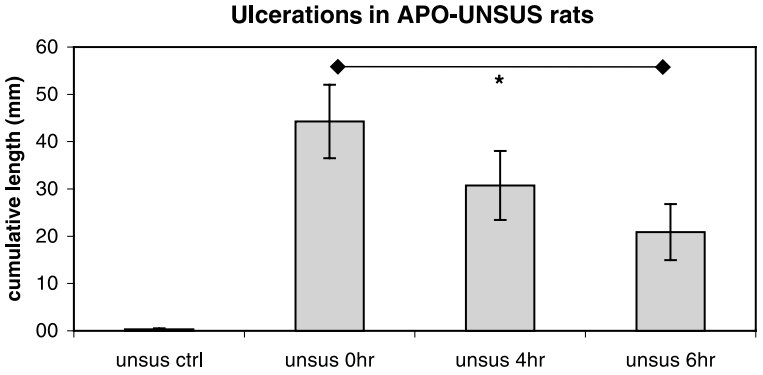


Fig. 2. Ulcerations (mm) in APO-UNSUS rats after 75 minutes of restraint-in-water stress (RIWS) (Mean ± SEM). RIWS produced ulcerations in all animals. Six hours after the RIWS animals had significantly less ulcerations than immediately after the stressor.

shown in Figs. 1 and 2 reveals that the degree of ulceration in APO-UNSUS rats (44 ± 8 mm) did not differ from that in APO-SUS rats (29 ± 9 mm), although the ulceration in the APO-UNSUS rats was slightly greater than that in APO-SUS rats.

## Discussion

The aim of the present study was to measure the effect of restraint-in-water-stress on the extent of ulceration in two neurobehavioural fundamentally different types of rats, the APO-SUS and APO-UNSUS lines. Unexpectedly, no difference in gastric ulceration was observed, a finding not in line with the results from Overmier et al. [24]. In the search for a factor that could predict ulcer vulnerability they subjected rats to six different (neuro)behavioural tests and found that the apomorphine test was the only paradigm to anticipate the susceptibility to develop ulcers. The greatest contribution came from the latency to initiate stereotypic gnawing, which accounted for 25% of the common variance with ulcer vulnerability. The other apomorphine variable they measured - APOTOT, a measure comparable to the one used in our selection procedure - also loaded highly on this ‘predictive’ factor. Based on these data we would have expected that APO-UNSUS rats show a high level of ulcerations and that APO-SUS rats show a low level of ulceration. This was not the case, although there was a slight tendency into the direction of that reported by Overmier et al. [24], namely somewhat more ulceration in the APO-UNSUS rats.

The following, not mutually exclusive, causes may explain the absence of the expected line difference in gastric ulcerations. First, there are differences in animals, design and apparatus between our and Overmier’s experiments. For instance, the latter group used Sprague Dawley rats, whereas we used Wistars. Also, the test apparatus in which the apomorphine sensitivity was determined varied, as well as the animals’ experimental history. Overmier’s animals were tested in 6 behavioural tests before their ulceration was measured, while ours were ‘naïve’. Although all these factors *per se* might not be decisive, together they might gain sufficient critical mass to explain the dissimilarities in results between our and Overmier’s experiments. Another explanation is that the stressor in this study, restraint-in-water-stress, is simply too severe for our animals. Previous experiments have shown that the differential stress

response between APO-SUS and APO-UNSUS is limited to mild and moderate stressors and that severe stressors result in similar - both behavioural, neuronal and endocrinological - responses [28].

A third explanation is more physiological and implies that the protective release of dopamine [5] counteracts the aggravating effects of corticosterone [18]. Hence, APO-SUS animals with more (protective) dopamine and more (destructive) corticosterone develop as many ulcerations as APO-UNSUS animals that have less dopamine and corticosterone.

The recovery, however, significantly differed between both types of rat: APO-SUS rats did not show any sign of recovery in contrast to APO-UNSUS rats that showed significantly less ulcerations 6 hours after the stressor than immediately afterwards. This finding can be explained by the fact that the stress-induced increase of corticosterone, which slows down the recovery form ulcerations [22], is both less and shorter-lasting in APO-UNSUS rats than in APO-SUS rats [12]. It also seems that the higher release of protective dopamine in APO-SUS is too short to compensate for the destructive actions of corticosterone. Similar to previously postulated explanations, future research is required to (in)validate this hypothesis. Given the present finding that the healing of the ulceration is line-specific, it is interesting to investigate whether the worsening of the RIWS induced ulceration that according to Overmier et al. [7] can occur during the first two hours after the RIWS, is also line-specific: for that purpose, the degree of ulceration needs to be analysed in the period of 60–90 min after the RIWS.

## Conclusion

In conclusion, this study provides evidence that the recovery from severe stressors, such as restraint-in-water stress, differs between APO-SUS and APO-UNSUS rats. Since APO-SUS and APO-UNSUS are the result of genetic selection, these data suggest that genetic factors are, at least partly, responsible for this recovery.

Because the APO-SUS rat is considered to be a valid model of certain aspects of schizophrenia [29], it becomes worthwhile to investigate whether patients with schizophrenia, once they suffer from ulcers, show a less rapid recovery of stomach ulcers than healthy subjects do. It is already known that the incidence of ulcers in these patients is less than in healthy subjects [5,24], a phenomenon that is understandable in view of the relatively high dopamine activity in their brain.

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