Chasing the Dragon Away: Personality as a protective factor and extended-release naltrexone as a treatment for heroin dependence
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Subchronic administration of short-acting naltrexone has no effect on striatal dopamine transporter availability, food intake or body weight gain in rats

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Abstract

The opioid receptor antagonist naltrexone is successfully used in the treatment of opioid and alcohol dependence. However, questions have been raised about possible anhedonic side effects, because the opioid system is directly involved in hedonic responses to natural rewarding activities, possibly due to its indirect effects on the striatal dopamine transporter (DAT). In order to test this hypothesis, 30 rats were randomized to either a 10-day treatment with 3 mg/kg short-acting naltrexone or vehicle. No significant differences between the groups were found in striatal DAT availability, cumulative food intake (for 48 or 72 h), body weight gain and abdominal fatpad weight. Thus, the results of this study suggest that (sub)chronic treatment with short-acting naltrexone does not induce possible anhedonic effects. However, it cannot be ruled out the anhedonic effect of naltrexone is only short-lived and thus not detected in the current study. Therefore, future studies are needed to study possible acute anhedonic effects at several time points shortly after short-acting naltrexone administration and to directly compare the possible anhedonic effects of long-acting with those of short-acting opioid antagonists.

Keywords: [$^{123}$I]FP-CIT, dopamine plasma membrane transport proteins, naltrexone, rats, corpus striatum, anhedonia
Introduction

The opioid receptor antagonist naltrexone is successfully used in the treatment of opioid and alcohol dependence (1,2). However, questions have been raised about its possible anhedonic side effects (3). Because the opioid system is directly involved in natural rewarding activities (4,5), it has been suggested that naltrexone blocks the hedonic responses to natural rewarding activities such as palatable food consumption in animals (6–11) and physical exercise, eating and sex in humans (12–14). Other studies found minimal or no effects on food intake in animals that previously had consumed only chow, whereas they did find a decreased chow intake in rats that had previously consumed palatable foods or fluids (15,16).

In humans, anhedonia and apathy are also associated with decreased striatal dopamine transporter (DAT) levels in the caudate nucleus and putamen (17), putamen (18) and caudate nucleus (19). Moreover, a study in rodents showed that subchronic blockade of opioid receptors for 7 days by (extended-release) naltrexone pellets resulted in a significant reduction of DAT expression in the striatum as a whole (20). This suggests that extended-release naltrexone may cause a downregulation of striatal DATs and subsequently anhedonia.

To improve our insights into the mechanism of action of short-acting naltrexone, the present study tested whether (sub)chronic treatment with short-acting naltrexone affects striatal DAT binding and associated responses to natural rewards, indicated by food intake, body weight gain and abdominal fatpad weight in rats. Nocturnal locomotor activity was measured to control for its possible confounding role in body weight gain.

Methods and materials

The effects of naltrexone on DAT binding were tested with the well-validated DAT tracer \textsuperscript{[123I]}FP-CIT (21). Based on the results of a power analysis ($\alpha=0.05$, $\beta=0.20$, expected specific striatal to non-specific (cerebellar) binding ratio of 2.7 with a SD of 0.08 (22), relevant difference 10%), 30 adult male Wistar rats (Harlan, Horst, The Netherlands; weighing 300 ± 20 g) were (sub)chronically treated with naltrexone or vehicle. They were individually housed in a temperature- (21-23°C) and humidity-controlled room with a 12-hour light/dark cycle (lights on at 7:00 am) with food and water available ad libitum. The rats were fed the local standard chow (Special Diet Services).

All experiments were approved by the Animal Ethics Committee (AMC, Amsterdam, The Netherlands).

Experimental design

Following a habituation period of 7 days, rats were randomly assigned to a treatment with naltrexone (NTX, $n=15$), or vehicle (VEH, $n=15$) for 10 consecutive days. The NTX group received 3 mg/kg naltrexone once daily between 9 and 10 am by
intraperitoneal injection. The naltrexone dose of 3 mg/kg was based on the doses used in previous studies that produced reductions in alcohol self-administration and/or relapse-like behaviors (23). Naltrexone was dissolved in 0.9% NaCl. The VEH group received a similar volume (0.1 mL/100 g) of 0.9% NaCl daily between 9 and 10 am by intraperitoneal injection. The solutions were prepared once a week, were kept out of direct UV light, and were refrigerated.

On the day of the last treatment dose, 6-8 h after the last injection, all animals were sacrificed for striatal DAT measurements (see below). After sacrifice, abdominal fatpads were dissected and weighted as described previously (24).

**Dopamine transporter measurement**

On the last day of the experiment, rats were anesthetized with ketamine/xylazine (ratio 2:1) followed by administration of approximately 40 MBq [¹²³I]FP-CIT intravenously via a lateral tail vein. [¹²³I]FP-CIT (GE Healthcare, Eindhoven, The Netherlands) had a specific activity of 750 MBq/nmol and a radiochemical purity > 95%. Two hours after injection (21), animals were sacrificed by bleeding through heart puncture under anesthesia and their brains were quickly removed, frozen on dry ice and sliced horizontally into 50 µm slices in a microtome cryostat at -21°C. To determine DAT availability in the dorsal striatum and nucleus accumbens, storage phosphor imaging, and analyses of the images, were performed as described previously (22,25).

**Behavioral measurements**

Food intake and body weight were measured three times a week for each animal separately. As our food intake measure we used the cumulative consumption of 48 or 72 h.

Nocturnal locomotor activity was recorded pre-treatment (days 4-6 of the habituation period) and during treatment (days 8-10), due to technical reasons in 24 of the 30 animals. A piezoelectronic stabilimeter was placed under the rat cage for 48 h. The nocturnal activity was measured in arbitrary units proportional to the voltage output. The average activity of the hours during the dark period (07:00 pm–07:00 am) was calculated as described earlier (24).

**Statistical analysis**

Data were tested for normality using the Kolmogorov-Smirnoff test. Independent samples t-tests and Mann-Whitney U tests were performed for differences between the NTX and VEH group.

The difference between the average nocturnal locomotor activity for 48 h before and during treatment was compared between the NTX and the VEH group.

Correlations between DAT availability and body weight gain, abdominal fatpad weight and food intake were determined with Pearson’s correlation coefficient for
normally distributed variables and with Spearman’s correlation coefficient for variables that were not normally distributed.

Because of the relatively small sample size, we reported both significance levels and standardized effect sizes ($d$-values; (26)) for each test, where $d=0.2$ is considered a small effect, $d=0.5$ a medium effect and $d=0.8$ a large effect. For the Mann-Whitney $U$ tests the effect size was calculated without outliers (>2 SD) to adjust for the skewed distribution.

All statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS) version 20. A probability value of less than 0.05 was considered significant. Exclusion of outliers (>2 SD difference from mean) did not affect the results.

**Results**

**DAT availability**

In four animals we were unable to determine DAT availability in the nucleus accumbens (1 NTX, 3 VEH) due to data acquisition failure. No significant between-group differences in DAT availability were found in the dorsal striatum ($t=0.018$, $p=0.985$, $d=-0.01$) and the ventral striatum/nucleus accumbens ($t=-1.191$, $p=0.245$, $d=0.47$; Table 1).

**Food intake, body weight gain, abdominal fatpad weight and plasma insulin concentrations**

Average food intake per day on day 7 (day before first injection) did not differ significantly between groups (NTX: 28.51 ± 9.1 (mean ± SD), VEH: 27.27 ± 10.0, $t=0.356$, $p=0.724$, $d=0.13$). Cumulative food intake data were missing for two animals (1 NTX, 1 VEH), due to data acquisition failure. Cumulative food intake did not differ significantly between groups (NTX: 387.82 ± 41.9, VEH: 379.42 ± 31.2, $t=0.600$, $p=0.554$, $d=0.23$; Figure 1).

The average body weights on day 2 of the experiment (adaptation period) did not differ significantly between groups (NTX: 301.73 ± 11.7 g, VEH: 300.73 ± 11.3 g, $t=0.238$, $p=0.814$, $d=0.09$). No significant differences between groups were found in

| Table 1. DAT availability (mean ± SD). |
|-----------------|-----------------|-----------------|
|                 | Dorsal striatum ($n$) | Nucleus accumbens ($n$) |
| NTX             | 4.41 ± 0.41 (15) | 2.98 ± 0.32 (14) |
| VEH             | 4.41 ± 0.57 (15) | 2.81 ± 0.38 (12) |

NTX: animals treated with naltrexone; VEH: animals treated with vehicle; $n$: number of animals in the analysis.
NALTREXONE HAS NO EFFECT ON DAT AVAILABILITY, FOOD INTAKE OR BODY WEIGHT GAIN IN RATS

Locomotor activity

Figure 1. Cumulative food intake (mean ± s.e.m.). Treatment period is marked. NTX: animals treated with naltrexone 3 mg/kg once daily; VEH: animals treated with vehicle.

Body weight curves

Figure 2. Body weight curves (mean ± s.e.m.). Treatment period is marked. NTX: animals treated with naltrexone 3 mg/kg once daily; VEH: animals treated with vehicle.

body weight gain between the day before first injection and the day before sacrifice (two outliers (1 NTX, 1 VEH); NTX: 38.20 ± 18.0 g, VEH: 38.47 ± 15.5 g, U=106.00, p=0.806, d=-0.08; Figure 2).

No significant differences between groups were found for abdominal fatpad weight (one outlier (VEH); NTX: 7.10 ± 1.1, VEH: 6.91 ± 2.1, U=88.00, p=0.325, d=0.43).

Locomotor activity

Data were missing for three animals (3 NTX), due to a defective stabilimeter. Nocturnal locomotor activity was not significantly different between groups (one
Association between behavioural data and DAT availability
There were no significant correlations between striatal DAT availability and food intake, body weight gain or abdominal fatpad weight.

Discussion
In rats, subchronic treatment with intraperitoneal injections of short-acting naltrexone had no effect on striatal DAT availability (both in dorsal and ventral parts of the striatum), food intake, body weight gain and abdominal fatpads. In addition, there were no significant correlations between DAT availability and food intake, body weight gain, and abdominal fatpad weight.

In contrast to our findings, Bhargava and co-workers reported a significant reduction in DAT expression in the striatum (up to 63%) after extended-release naltrexone administration using 10 mg pellets during 7 days when the pellets were not removed before sacrificing (20). However, when the pellets were removed 16 h before sacrificing, there was no significant effect on striatal DATs (20). In the present study, we used short-acting naltrexone, and the animals were sacrificed 6-8 h after the last drug administration; a situation very comparable with the condition in which the pellets were removed 16 h before sacrificing.

Although we did not find any changes in DAT binding ($B_{\text{max}}$), it cannot be excluded that there were changes in DAT reuptake ($V_{\text{max}}$) because of differences in dopamine release (27). In addition, in the present study we measured striatal DAT availability 6-8 h after the last administration of naltrexone. However, the half-life of naltrexone is around 2.7 h (28), and therefore we cannot rule out the possibility that the indirect inhibitory effect of naltrexone on dopamine release had ceased and dopamine levels had returned to normal levels at 6-8 h after the last administration of naltrexone. However, although we cannot completely exclude that effects on DAT availability do exist 2-3 h, but not 6-8 h after the last injection of naltrexone, previous studies in non-human primates showed that acute changes in dopamine concentrations (e.g. acute dopamine depletion) did not influence striatal DAT binding in vivo (29). Moreover, DAT expression and naltrexone effects on food intake may be higher in females than males (30); therefore, it may be of interest if our present findings in male rats could be reproduced in female rats.

In addition, in future studies it may be of interest to directly compare the effects of short-acting naltrexone and extended-release naltrexone (which produces more stable plasma concentrations than single doses) on DAT availability, preferably both in small laboratory animals and in humans using both parenteral and oral routes of administration.
Apart from the lack of an effect on DAT availability, we also found no significant effects of subchronic administration of short-acting naltrexone on other markers of hedonia; food intake, body weight gain, and abdominal fatpad weight did not change after naltrexone administration. Our findings are in line with the literature, because our rats were offered only chow. In future studies it may be of interest to also study the effects of naltrexone in animals that are fed not with standard chow, but with a palatable diet, because chow consumption may be driven by metabolic needs and not necessarily be reflective of reward functions.

Interestingly, Barrios De Tomasi and Juárez showed that the food intake decreased 2-4 h after administration of a single, but high, dose of naltrexone (10 mg/kg), but not at lower dosages (31). In the present study, however, the cumulative food intake was measured for 48 or 72 h. Therefore, it cannot be ruled out that an effect on food intake was present immediately after the naltrexone injections, and thus future studies should also measure food intake shortly after naltrexone injection. Furthermore, all rats in this study were housed individually which can lead to stress reactions including changes in eating behavior, stress hormones and cytokine production (32), and this may interfere with naltrexone treatment. However, if such an effect has occurred it was a systematic effect affecting all animals under study, i.e. both animals with naltrexone and animals with vehicle injections.

Human studies are inconsistent with regard to the anhedonic effect of opioid antagonists. Daniel and co-workers showed that exercise in a high-intensity aerobics class induced significant positive changes in mood, which did not occur when participants were pre-treated with 50 mg short-acting naltrexone (12). Murphy and co-workers showed that one dose of naloxone induced a decrease in the level of oxytocin and subjective arousal and pleasure at orgasm (13). In a review, Yeomans and Gray (14) found that 14 of the 17 controlled studies in normal-weight or obese humans showed a decrease in food intake after one dose of an opioid antagonist. However, a recent survey by O’Brien et al. (33) showed that chronic treatment of alcohol dependence with extended-release naltrexone did not result in anhedonia. These inconsistent results may be caused by differences in study design (e.g. single dose vs. long-term treatment; with or without control group), compound or formulation that was tested (e.g. short-acting naltrexone/naloxone vs. extended-release naltrexone), and the measurement of anhedonia.

The current study has both strengths and weaknesses. An important strength is that we were able to show that nocturnal locomotor activity was not associated with our anhedonia measures and that our results are thus not confounded by this process. The most important limitations are that we did not use different dosages and treatment durations of short-acting naltrexone, did not administer naltrexone in the active ‘dark’ cycle, did not measure food intake at several time points during the day, and that the study was not performed in rats that were previously exposed to heroin. We cannot exclude that longer (chronic) treatment duration results in
effects on food intake and DAT availability. Also, the stress of daily injection may have confounded the findings.

**Conclusion**

(Sub)chronic treatment with short-acting naltrexone does not lead to a decrease in striatal DAT availability, cumulative food intake (for 48 or 72 h) and body weight gain in rats. Thus, the results of this study suggest that (sub)chronic treatment with short-acting naltrexone does not induce possible anhedonic effects. However, it cannot be ruled out the anhedonic effect of naltrexone are only short-lived and thus not detected in the current study. Therefore, future studies are needed to study possible acute anhedonic effects at several time points shortly after short-acting naltrexone administration and to directly compare the possible anhedonic effects of long-acting with those of short-acting opioid antagonists.

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**Author contributions**

Authors Jan Booij, Wim van den Brink, Eline R. Zaaijer and Susanne E. la Fleur designed the study and wrote the protocol. Author Eline R. Zaaijer, Jan Booij and Kora de Bruin managed the literature searches and analyses. Author Eline R. Zaaijer undertook the statistical analysis, and author Eline R. Zaaijer wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.
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