REM Sleep Deprivation Potentiates the Effects of Imipramine and Desipramine but Not That of Clomipramine in the Forced Swimming Test

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ABSTRACT—Effects of REM sleep (REMs) deprivation on the basal swimming activity and the tricyclic antidepressants-induced increase in swimming activity in the forced swimming test were investigated. Immediately after a 48-hr period of REMs deprivation, the basal swimming activity in REMs-deprived mice was significantly higher than those in group-housed and socially isolated animals used as the control groups. The REMs deprivation-induced increase in the swimming activity was not changed by adrenoceptor antagonists and it returned to the control levels 3 hr after the REMs deprivation treatment. Moreover, imipramine and desipramine but not clomipramine further increased the swimming activity enhanced by REMs deprivation at doses that did not affect the activity in the control groups. The enhancing effect of REMs deprivation on the sensitivity to imipramine and desipramine remained unchanged even at 3 hr after the REMs deprivation treatment, and it was blocked by the α_2-adrenoceptor antagonist yohimbine. These results suggest that the REMs deprivation-induced increase in basal swimming activity in the forced swimming test is not mediated by adrenoceptor mechanisms, whereas the enhancing effect of REMs deprivation on the sensitivity to imipramine and desipramine may be mediated by the functional changes in α_2-adrenoceptors in the brain.

Keywords: REM sleep deprivation, Forced swimming test, Imipramine, Desipramine, Clomipramine

REM sleep (REMs) deprivation has been clinically shown to improve certain types of depression in humans (for a review, see ref. 1). In rodents, this treatment decreases the immobility time (or increases the swimming activity) in the forced swimming test (2–6), a primary method for screening antidepressant drugs originally introduced by Porsolt et al. (7). REMs deprivation seems to have similar effects to those of tricyclic antidepressant drugs on the central noradrenergic system in other behavioral and neurochemical experiments. REMs deprivation as well as chronic antidepressant drug treatment attenuates the sedation induced by clonidine, an α_2-adrenoceptor agonist (8; for a review, see ref. 9). In in vitro studies, REMs deprivation treatment reduced the accumulation of cyclic AMP stimulated by noradrenaline and decreased the density of β-adrenoceptors in the cortex of rats (for a review, see ref. 10). These neurochemical changes induced by REMs deprivation have also been demonstrated in the rats pretreated with antidepressant drugs (for a review, see ref. 9). Therefore, these similarities between the effects of REMs deprivation and those of chronic antidepressants raise the possibility that the antidepressants-like effect of REMs deprivation in the forced swimming test may be mediated by the same mechanism(s) as those of antidepressant drugs, and that combination of REMs deprivation with antidepressant drug treatment may modify the effects of antidepressant drugs.

We undertook the present study to determine whether an adrenoceptor mechanism is commonly involved in the REMs deprivation- and the tricyclic antidepressant drugs-induced increase in swimming activity in the forced swimming test. Furthermore, to clarify whether REMs deprivation modifies the effect of an antidepressant drug, we tested the effects of REMs deprivation on the imipramine-, desipramine- and clomipramine-induced increase in swimming activity.

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MATERIALS AND METHODS

Animals

Male 5-week-old ddY mice (Japan SLC, Inc., Hamamatsu) were used in the experiments. The animals were housed in groups of 20–25 per cage (35 × 30 × 16 cm), for at least 1 week before the start of the experiment, with free access to food and water. Housing conditions were thermostatically maintained at 24 ± 1 ℃, with a 12-hr light/dark cycle (light on 07 : 30–19 : 30).

REM sleep deprivation

Mice were deprived of REMs by the small pedestal (platform) method as described previously (6, 11). In brief, a small pedestal (4.5-cm-high, 1.8 cm in diameter) was fixed at the center of a REMs deprivation chamber (20 × 15 × 21 cm) and was surrounded by water (3.5-cm-deep). A group of mice was placed individually in the chamber and housed for 48 hr with free access to food and water (REMs-deprived mice). During the REMs deprivation period, other groups of mice were either housed in groups of four (group-housed mice) or housed individually (isolated mice) in a Plexiglas cage (25 × 18 × 12 cm), and they were used as the control groups. After the termination of REMs deprivation, each mouse was placed individually in the Plexiglas cage for 3 hr, with the exception of the animals used for immediate experimentation.

The forced swimming test

Each mouse was placed individually in a transparent glass cylinder (20-cm-high, 8 cm in diameter) containing fresh water (25 ℃, 8-cm-deep) and was forced to swim for 15 min (pretest swimming). After a 20-min drying period, the animals were deprived of REMs for 48 hr. Immediately after the termination of REMs deprivation or after a 3-hr recovery period, the animals were placed in the cylinder for 5 min (test swimming). Swimming activity during the test was measured using an animal movement analyzing system, Scanet SV-10 (Toyo Sangyo Co., Ltd., Toyama), as described previously (6). In brief, this system consisted of a rectangular enclosure (40 × 38 cm), the side walls (12 cm) of which were equipped with 144 pairs of photosensors. Each pair of photosensors was set at a height of 8.8 cm above the floor, and was scanned every 0.1 sec to detect animal movement. Swimming activity was calculated from the scanning data obtained.

Drugs

Phentolamine mesylate (Regitine® Inj.; Japan Ciba-Geigy, Ltd., Takarazuka), yohimbine HCl and DL-propranolol HCl (Nacalai Tesque, Inc., Kyoto) were administered at 1 hr prior to testing. Imipramine HCl, desipramine HCl, and clomipramine HCl (Sigma Chemical Co., St. Louis, MO, USA) were given immediately after the pretest swimming and at 24, 5 and 1 hr before the test swimming. All drugs were dissolved in or diluted with saline just before the experiments and were intraperitoneally injected using a constant volume (0.01 ml/g body weight).

Statistical analysis

Swimming activity in the test swimming was analyzed by one-way analysis of variance (ANOVA) followed by the two-tailed multiple Student's *t*-test. Differences of $P < 0.05$ were considered significant.

RESULTS

The effects of adrenoceptor antagonists on the REMs deprivation- and tricyclic antidepressant drugs-induced increase in swimming activity

As previously reported (6), when tested immediately

Table 1. The effects of adrenoceptor antagonists on the REM sleep deprivation-induced increase in swimming activity

Drugs	Doses	Swimming activity (counts/5 min)	
Yohimbine HCl	(mg/kg)		
Group-housed	0	505 ± 37	(16)
Isolated	0	534 ± 60	(16)
REMs-deprived	0	754 ± 63** ##	(16)
REMs-deprived	5	774 ± 55$^{N.S.}$	(15)
Phentolamine mesylate	(mg/kg)		
Group-housed	0	492 ± 39	(12)
Isolated	0	520 ± 64	(12)
REMs-deprived	0	901 ± 97** ##	(12)
REMs-deprived	5	827 ± 115$^{N.S.}$	(12)
REMs-deprived	10	868 ± 80$^{N.S.}$	(12)
REMs-deprived	20	1086 ± 109$^{N.S.}$	(12)
DL-Propranolol HCl	(mg/kg)		
Group-housed	0	519 ± 80	(12)
Isolated	0	566 ± 80	(12)
REMs-deprived	0	918 ± 88** #	(12)
REMs-deprived	5	907 ± 114$^{N.S.}$	(12)
REMs-deprived	10	1103 ± 135$^{N.S.}$	(12)
REMs-deprived	30	1018 ± 87$^{N.S.}$	(12)

Mice were either group housed, socially isolated, or deprived of REM sleep (REMs). Immediately after the termination of REMs deprivation, swimming activity was measured. Yohimbine HCl, phentolamine mesylate, or DL-propranolol HCl was injected 1 hr before the test swimming. Each value represents the mean ± S.E.M. Numbers in parentheses indicate the number of animals. $^{**}P < 0.01$, compared to group-housed animals; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared to isolated animals; $^{N.S.}P > 0.05$ compared to REMs-deprived control animals (multiple Student's *t*-test).

after the termination of REMs deprivation treatment, a significant increase in the basal swimming activity was observed in REMs-deprived mice. The REMs deprivation-induced increase in basal swimming activity was not changed by yohimbine, an α_2-adrenoceptor antagonist; phentolamine (5, 10, and 20 mg/kg), an α-adrenoceptor antagonist; or propranolol (5, 10, and 30 mg/kg), a β-adrenoceptor antagonist (Table 1).

Repeated administration of imipramine (20, 30, and 40 mg/kg, ×4), a noradrenaline and serotonin uptake blocker, and desipramine (10, 20, and 30 mg/kg, ×4), a selective noradrenaline uptake blocker, dose-dependently increased the swimming activity in the group-housed control mice. The dose-response curve of clomipramine (20, 30, and 40 mg/kg, ×4), a preferential serotonin uptake blocker, was bell-shaped; and the effect of this drug was statistically significant only at a dose of 30 mg/kg (Fig. 1). Yohimbine (5 mg/kg) significantly decreased the swimming activity enhanced by imipramine (40 mg/kg, ×4) and desipramine (30 mg/kg, ×4) in the group-housed control animals. However, this antagonist did not affect the clomipramine (30 mg/kg, ×4)-induced increase in swimming activity (Table 2).

The effects of imipramine, desipramine, and clomipramine on the swimming activity in REMs-deprived mice

When tested immediately after REMs deprivation treatment, repeated administration of imipramine (20 mg/kg, ×4) further increased the basal swimming activity enhanced by REMs deprivation, while it did not affect the activity in the group-housed or isolated animals (Fig. 2A). Desipramine (10 mg/kg, ×4) also significantly increased the swimming activity in the REMs-deprived mice at a dose that did not affect the activity in the control groups (Fig. 2B).

Three hours after the termination of REMs deprivation

Table 2. The effect of yohimbine on the antidepressant drug-induced increase in swimming activity in group-housed control mice

Drugs	(mg/kg, ×4)	Swimming activity (counts/5 min) Saline	Yohimbine HCl
Imipramine HCl	0	515± 38	441±34
	40	1126± 80**	830±79##
Desipramine HCl	0	506± 43	571±58
	30	1063± 70**	843±78#
Clomipramine HCl	0	419± 36	470±88
	30	746±114**	633±68N.S.

Mice were group housed before the test swimming; and imipramine HCl, desipramine HCl, or clomipramine HCl was administered 4 times before the test swimming as described in the text. Yohimbine HCl (5 mg/kg) was administered at 1 hr before the test swimming. Each value represents the mean swimming activity obtained from 16 animals, with the S.E.M. indicated. **$P<0.01$, compared to the respective saline control animals; #$P<0.05$, ##$P<0.01$, compared to the mice treated with antidepressant drugs alone; N.S.$P>0.05$, compared to the mice treated with antidepressant drugs alone (multiple Student's t-test).

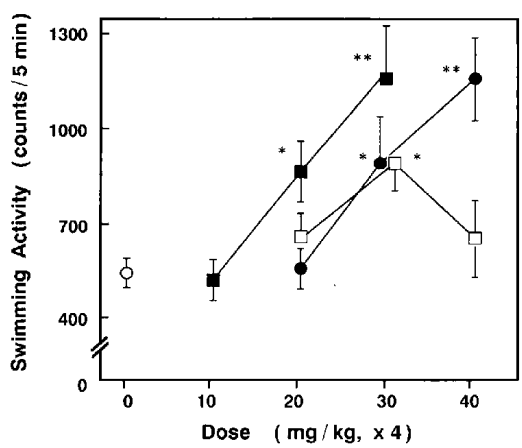

Fig. 1. The effects of repeated administration of antidepressant drugs on swimming activity in the group-housed control. Mice were group housed before the test swimming; and imipramine HCl (●), desipramine HCl (■), or clomipramine HCl (□) was administered 4 times as described in the text. Each point represents the mean swimming activity obtained from 12–16 animals, the vertical bar representing the S.E.M. *$P<0.05$, **$P<0.01$, compared to the saline control animals (○) (multiple Student's t-test).

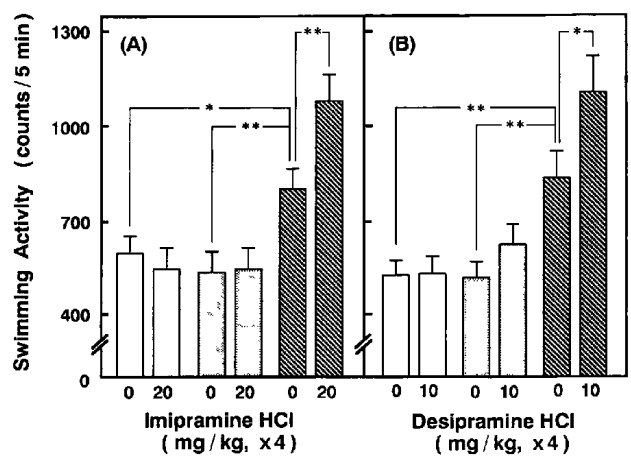

Fig. 2. The effects of imipramine and desipramine on swimming activity in REM sleep-deprived mice. Mice were either group housed (open column), socially isolated (dotted column), or deprived of REM sleep (hatched column). Immediately after the termination of REM sleep deprivation, swimming activity was measured. (A) Imipramine HCl (20 mg/kg) or (B) desipramine HCl (10 mg/kg) was administered 4 times before the test swimming. Each bar height represents the mean±S.E.M. obtained from 15 or 16 animals. *$P<0.05$, **$P<0.01$, compared to each group (multiple Student's t-test).

treatment, the basal swimming activity in the REMs-deprived mice returned to the control levels, but repeated administration of imipramine (20 mg/kg, ×4) and desipramine (10 mg/kg, ×4) still significantly increased the swimming activity in REMs-deprived mice (Fig. 3, A and B).

Clomipramine at a dose of 20 mg/kg, ×4 did not increase the swimming activity in REMs-deprived mice, whereas at a higher dose (30 mg/kg, ×4), this drug significantly increased the activity in REMs-deprived animals. However, there was no significant difference in the latter effect between REMs-deprived animals and the control groups (Figs. 4 and 5).

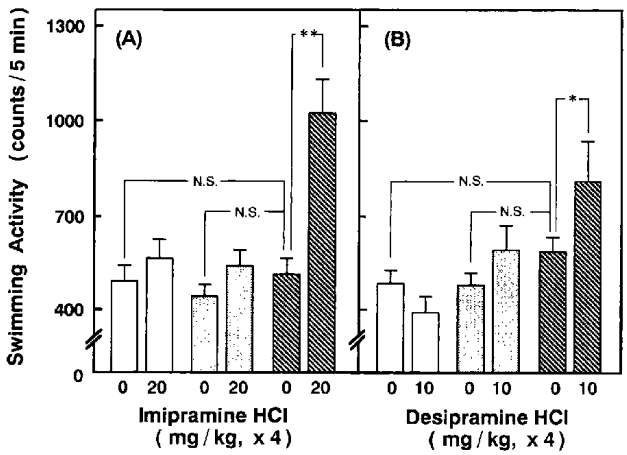

Fig. 3. The effects of imipramine and desipramine on swimming activity in REM sleep-deprived mice. Mice were either group housed (open column), socially isolated (dotted column), or deprived of REM sleep (hatched column). Three hours after the termination of REM sleep deprivation, swimming activity was measured. (A) Imipramine HCl (20 mg/kg) or (B) desipramine HCl (10 mg/kg) was administered 4 times before the test swimming. Each bar height represents the mean±S.E.M. obtained from 15 or 16 animals. $^{*}P<0.05$, $^{**}P<0.01$, compared to each group; $^{N.S.}P>0.05$, compared to the saline-treated group-housed and isolated animals (multiple Student's *t*-test).

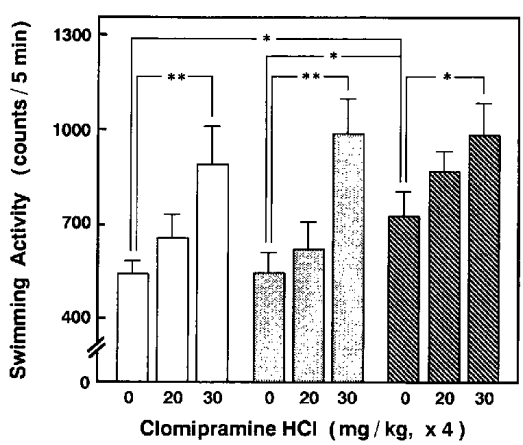

Fig. 4. The effect of clomipramine on swimming activity in REM sleep-deprived mice. Mice were either group housed (open column), socially isolated (dotted column), or deprived of REM sleep (hatched column). Immediately after the termination of REM sleep deprivation, swimming activity was measured. Clomipramine HCl (20 and 30 mg/kg) was administered 4 times before the test swimming as described in the text. Each bar represents the mean±S.E.M. obtained from 16 animals. $^{*}P<0.05$, $^{**}P<0.01$, compared to each group (multiple Student's *t*-test).

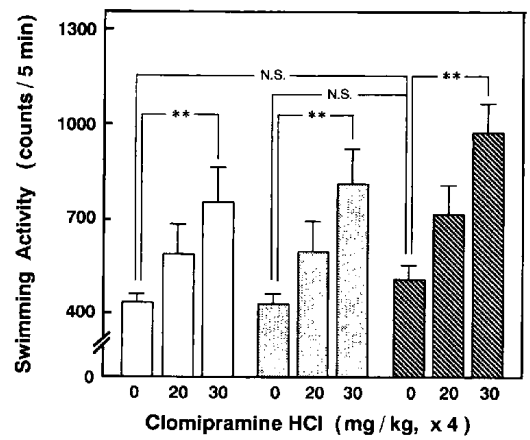

Fig. 5. The effect of clomipramine on swimming activity in REM sleep-deprived mice. Mice were either group housed (open column), socially isolated (dotted column), or deprived of REM sleep (hatched column). Three hours after the termination of REM sleep deprivation, swimming activity was measured. Clomipramine HCl (20 and 30 mg/kg) was administered 4 times before the test swimming. Each bar represents the mean±S.E.M. obtained from 16 animals. $^{**}P<0.01$, compared to each group; $^{N.S.}P>0.05$, compared to the saline-treated group-housed and isolated animals (multiple Student's *t*-test).

Table 3. The effect of yohimbine on the imipramine- and desipramine-induced increase in swimming activity in REM sleep-deprived mice

Drugs	(mg/kg, ×4)	Swimming activity (counts/5 min) Saline	Yohimbine HCl
Imipramine HCl	0	521± 49	485±45
	20	754± 87*	554±56#
Desipramine HCl	0	524± 41	502±47
	10	904±109**	679±61#

Mice were deprived of REM sleep. Three hours after the termination of REM sleep deprivation, swimming activity was measured. Imipramine HCl or desipramine HCl was administered 4 times before the test swimming as described in the text. Yohimbine HCl (5 mg/kg) was administered at 1 hr before the testing. Each value represents the mean swimming activity obtained from 14–16 animals, with the S.E.M. indicated. $^{*}P<0.05$, $^{**}P<0.01$, compared to the saline control animals; $^{\#}P<0.05$, compared to the mice treated with antidepressant drug alone (multiple Student's *t*-test).

Table 4. The effect of propranolol on the desipramine-induced increase in swimming activity in REM sleep-deprived mice

Drugs (mg/kg)		Swimming activity (counts/5 min)	
		Saline	Desipramine HCl
Propranolol HCl	0	528± 54	1033±152**
	5	553± 47	1151± 93$^{N.S.}$
	10	656±107	1192± 79$^{N.S.}$
	30	659± 85	1177± 87$^{N.S.}$

Mice were deprived of REM sleep. Three hours after the termination of REM sleep deprivation, swimming activity was measured. Desipramine HCl (10 mg/kg) was administered 4 times before the test swimming as described in the text. DL-Propranolol HCl (5, 10, and 30 mg/kg) was administered at 1 hr before the testing. Each value represents the mean swimming activity obtained from 12–14 animals, with the S.E.M. indicated. **$P<0.01$, compared to the saline control animals; $^{N.S.}P>0.05$, compared to the mice treated with desipramine alone (multiple Student's *t*-test).

The effects of yohimbine and propranolol on the imipramine- and desipramine-induced increase in swimming activity in REM sleep-deprived mice

Yohimbine (5 mg/kg) did not change the basal swimming activity when examined 3 hr after the REMs deprivation treatment. This antagonist significantly blocked the effects of imipramine (20 mg/kg, ×4) and desipramine (10 mg/kg, ×4) on the swimming activity in REMs-deprived mice (Table 3). On the other hand, propranolol (5, 10, and 30 mg/kg) did not affect either the basal swimming activity or the desipramine (10 mg/kg, ×4)-induced increase in the swimming activity in REMs-deprived mice (Table 4).

DISCUSSION

As previously reported (6), the basal swimming activity in REMs-deprived mice was significantly higher than those in group-housed and isolated control animals, when examined immediately after the termination of REMs derivation. Since stimulation of adrenoceptors has been reported to play an important role in the anti-immobility effect in the forced swimming test (6, 12–15), it is possible that REMs deprivation may increase the tonic activity of the noradrenergic system in the brain, resulting in an increase in the basal swimming activity. However, this does not seem to be the case, because in the present study, neither phentolamine (α-adrenoceptor antagonist), yohimbine (α_2-adrenoceptor antagonist) nor propranolol (β-adrenoceptor antagonist) changed the basal swimming activity enhanced by REMs deprivation. The present findings are in contrast to the data reported by Van Luijtelaar and Coenen (5) that phentolamine (10 mg/kg) prevents the REMs deprivation-induced decrease in immobility time in the forced swimming test. The reasons for the discrepancy between their data and our results remain unclear, but it may be due to the differences in animal species, REMs deprivation method, injection and test timing, and/or method to detect animal behavior. On the other hand, the tricyclic antidepressant drugs used in the present study also significantly increased the swimming activity in the group-housed control mice. Consistent with the data demonstrating the involvement of an α_2-adrenoceptor mechanism in the anti-immobility effect of desipramine (16), the effects of imipramine (40 mg/kg, ×4), a noradrenaline and serotonin uptake blocker, and desipramine (30 mg/kg, ×4), a selective noradrenaline uptake blocker, but not of clomipramine (30 mg/kg, ×4), a preferential serotonin uptake blocker, on the swimming activity in group-housed control animals were blocked by yohimbine. Therefore, the present findings indicate that the mechanisms of the increase in swimming activity induced by REMs deprivation and clomipramine are different from those induced by imipramine and desipramine.

In the REMs-deprived mice, repeated administration of imipramine and desipramine further increased the basal swimming activity at the doses that did not change the swimming activity in the control groups. It should be noted that such enhancing effects of REMs deprivation was long-lasting and remained unchanged for at least 3 hr, whereas the effect of REMs deprivation on the basal swimming activity was short-lasting, and it had already disappeared by the time the animals were examined at 3 hr after the termination of REMs deprivation treatment. These results indicate that: 1) REMs deprivation potentiates the effects of imipramine and desipramine on the swimming activity in the forced swimming test, and 2) the mechanism of the increase in basal swimming activity by REMs deprivation can be experimentally distinguished from those of hypersensitivity to imipramine and desipramine in REMs-deprived mice.

At least two possibilities could account for the increase in imipramine and desipramine sensitivity by REMs deprivation. First, REMs deprivation may cause pharmacokinetic changes that result in the apparent increase of the sensitivity to imipramine and desipramine. In fact, stressful manipulations have been reported to affect drug metabolism (17, 18). However, this possibility seems unlikely, since REMs deprivation did not change the effective dose of clomipramine that is metabolized by the same pathway as imipramine (19). Secondly, REMs deprivation may induce the functional changes in the central nervous system in the mouse brain. In the present study, yohimbine inhibited the REMs deprivation-induced increase in the sensitivity to imipramine and desipramine, suggesting that REMs deprivation causes functional changes in brain α_2-adrenoceptors. This idea seems to be

supported by our previous data that REMs deprivation enhances the clonidine (α_2-adrenoceptor agonist)-induced increase in swimming activity in the forced swimming test (6). On the other hand, a β-adrenoceptor mechanism has been shown to be involved in the anti-immobility effects of antidepressant drugs such as desipramine and amitriptyline (20, 21). However, a single administration of propranolol failed to inhibit the effect of desipramine (10 mg/kg, $\times 4$) on swimming activity in REMs-deprived mice, suggesting that a β-adrenoceptor mechanism is not involved in the REMs deprivation-induced increase in desipramine sensitivity.

In conclusion, the present data indicate that REMs deprivation increases the basal swimming activity and potentiates the effect of imipramine and desipramine on swimming activity. The former effect is short-lasting and mediated by non-adrenoceptor mechanisms, whereas the latter is long-lasting and mediated by the REMs deprivation-induced functional change in α_2-adrenoceptors.

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