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Deep brain stimulation of the orbitofrontal cortex reduces perseverative behavior induced by quinpirole in rats

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Abstract

Introduction: *Different rodent models of obsessive-compulsive disorder (OCD) have been used with the purpose for understanding this mental disease; in a previous article, we described quinpirole (QP) as a dopaminergic inductor of perseverative behavior.* **Objective:** *The objective of this study was to evaluate the decrease of QP-induced perseverative behavior observed in an open field after unilateral deep brain stimulation-low frequency (DBS-LF) into the orbitofrontal cortex (OFC) in Wistar rats.* **Materials and Methods:** *A total of 40 adult male Wistar rats were divided into three groups, each group underwent pharmacological treatment: sodium chloride (NaCl), QP, and clomipramine (CMP), these groups were not implanted with an electrode. An additional group was implanted into OFC Sham (SH), and after behavior evaluation, unilateral DBS-LF (6 Hz) was applied during night time for 5 min each side; first left side and after right side.* **Results:** *Distance traveled significantly decreased in the 5 min observed between QP and NaCl (p = 0.016), and a significant difference was found between NaCl and SH (p = 0.020). There was no significant difference between NaCl and CMP (p = 0.582) or NaCl and DBS-LF (p = 0.829). No significant difference between DBS left side and right side was found (p = 1.00).* **Conclusions:** *Unilateral DBS of the OFC prevents perseverative behavior in an open field, induced by QP.*

Key words: *Deep brain stimulation. Quinpirole. Orbitofrontal cortex. Perseverative behavior.*

Introduction

The obsessive-compulsive disorder (OCD) rodent model can be pharmacologically induced with the following drugs, i.e., RU24969, 8-OH-DPAT, neonatal clomipramine (CMP), and quinpirole (QP)[1]. The OCD is compared to perseverative behavior in rats and is evaluated through an open field and a T-maze test. QP is a D2/D3 dopamine receptor agonist[1], and when chronically applied in rats, it can induce perseverative behavior. This effect is a model of OCD[2]. Dopamine induces motor functions in different regions of the central nervous system, involving different brain circuits. The rats motor activity decreases through the activation of the D2 receptors in striatopallidal neurons[3]. Repeated administration of dopaminergic D2 agonists produces receptor hypersensitivity, which conditions a receptor state known as high-affinity (D2High). This mechanism is responsible for some behaviors generated after administering QP[4]. Receptor hypersensitization is known to occur in some psychiatric disorders such as schizophrenia, OCD, and motor dyskinesias[4]. Similar behaviors are observed in animals sensitized with QP, such as stereotypies, antinociception, locomotor

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activity, alterations in memory, and learning. QP can decrease locomotor activity in the first 15 min after it is administered and it increases motor activity after the 2nd h of application[3].

Dvorkin, in 2010, suggested that QP may drive the vigor of the checking behavior by inhibition of nucleus accumbens neurons, and it can be a site for the negative feedback control of checking[5]; nevertheless, the mechanistic understanding of cellular and molecular changes occurring in the orbitofrontal cortex (OFC) is unclear[6]. Recent studies have been published using optogenetics to examine the contribution of OFC inputs to the striatum, in the evolution of perseverative grooming[7,8]; however, the pathologic changes that may alter these inputs in OCD are unknown[6].

de Haas et al. observed an increase of dopamine into the nucleus accumbens in perseverative rats treated with QP[9]. Schmidt and colleagues found that chronic treatment with QP produces perseverative behavior and increment of locomotion in rats; these findings were not observed in the control group where D-amphetamine was administered[10].

In 2007, McCracken and Grace applied deep brain stimulation (DBS) high frequency (130 Hz) into the OFC decreasing OCD behavior by reducing the neuronal activity and inhibiting the activation of the antidromic conduction of collateral axons in the corticostriatal region[11]. These findings suggest that using high frequency in the subthalamic nucleus can be used as a therapeutic strategy for OCD[12]. In addition, high-frequency DBS of the entopeduncular nucleus and globus pallidus decreases perseverative behavior in rats[13]. Previously, we have published information about lesion or low-frequency DBS into reticular nucleus of thalamus and how it decreases perseverative behavior in rats induced by 8OH-DPAT[14].

On the other hand, different anatomical structures such as the anterior limb of the internal capsule, nucleus accumbens, subthalamic nucleus, ventral striatum, and the inferior thalamic peduncle have been involved in the treatment of OCD patients[15,16]. Fitzgerald and Segrave suggest that DBS in the nucleus accumbens or ventral striatum could modulate the amygdala - basal ganglia - prefrontal circuitry that is abnormally active in mood and anxiety disorders[17]. Sturm, in 2003, reported that unilateral high-frequency DBS into accumbens nucleus could decrease OCD symptoms[18] that information differs to the rest of the authors who had applied bilateral DBS. Our team had published the decreasing effect of bilateral DBS of inferior thalamic peduncle in OCD symptoms of eight patients[15,19].

The aim of this study is to evaluate the effect of DBS low-frequency (DBS-LF) bilateral on OFC in Wistar rats with perseverative behavior induced by QP through an open field.

Materials and methods

Subjects

To perform this study, healthy male Wistar rats that weight between 250 and 350 g were selected, none of which showed abnormal motor behavior by rotarod test. Rats had free access to food and water, they were single housed and kept in a 12:12 light-dark cycle at a temperature-controlled room; to avoid bias caused by the circadian cycle of rats, DBS was performed at night[20]. The entire management of animals was done according to the Mexican Official Norm NOM-062-ZOO-1999. This experiment was approved by the Research and Ethics Committee of the *General Hospital of México "Dr. Eduardo Liceaga"*.

48 rats were included in the experiment; however, eight rats met the exclusion criteria: infection at the surgical site after electrode implantation (four rats), death during the experimental maneuver (two rats), and rats which ripped off the electrode (two rats).

Drugs

All groups of rats were induced to perseverative behavior by administering QP hydrochloride (Sigma-Aldrich by Eli Lilly and Company, Indianapolis, USA) chronically (0.5 mg/kg twice weekly for 5 weeks) subcutaneously at the cross-skull region, with the exception, of the sodium chloride (NaCl) group, which was injected with the same amount of the volume of 0.9% NaCl solution. For the surgical implantation of the electrode, the animals were placed in a stereotactic apparatus Kopf instruments, model 960 (David Kopf Instruments Tujunga Ca. USA).

Surgery was performed under an anesthetic procedure using Ketamine (Anesket, PISA Mexico) 0.1 mg/kg administered intramuscularly and Acepromazine (Calmivet, Vétoquinol, France) 0.5 mg/kg administered intramuscularly; no analgesic or antibiotic was administered to avoid bias caused by drug interaction.

Apparatus

Perseverative behavior was correlated with motor displacement (distance traveled in centimeters [cm]) by the rat, the further distance traveled the least perseverative

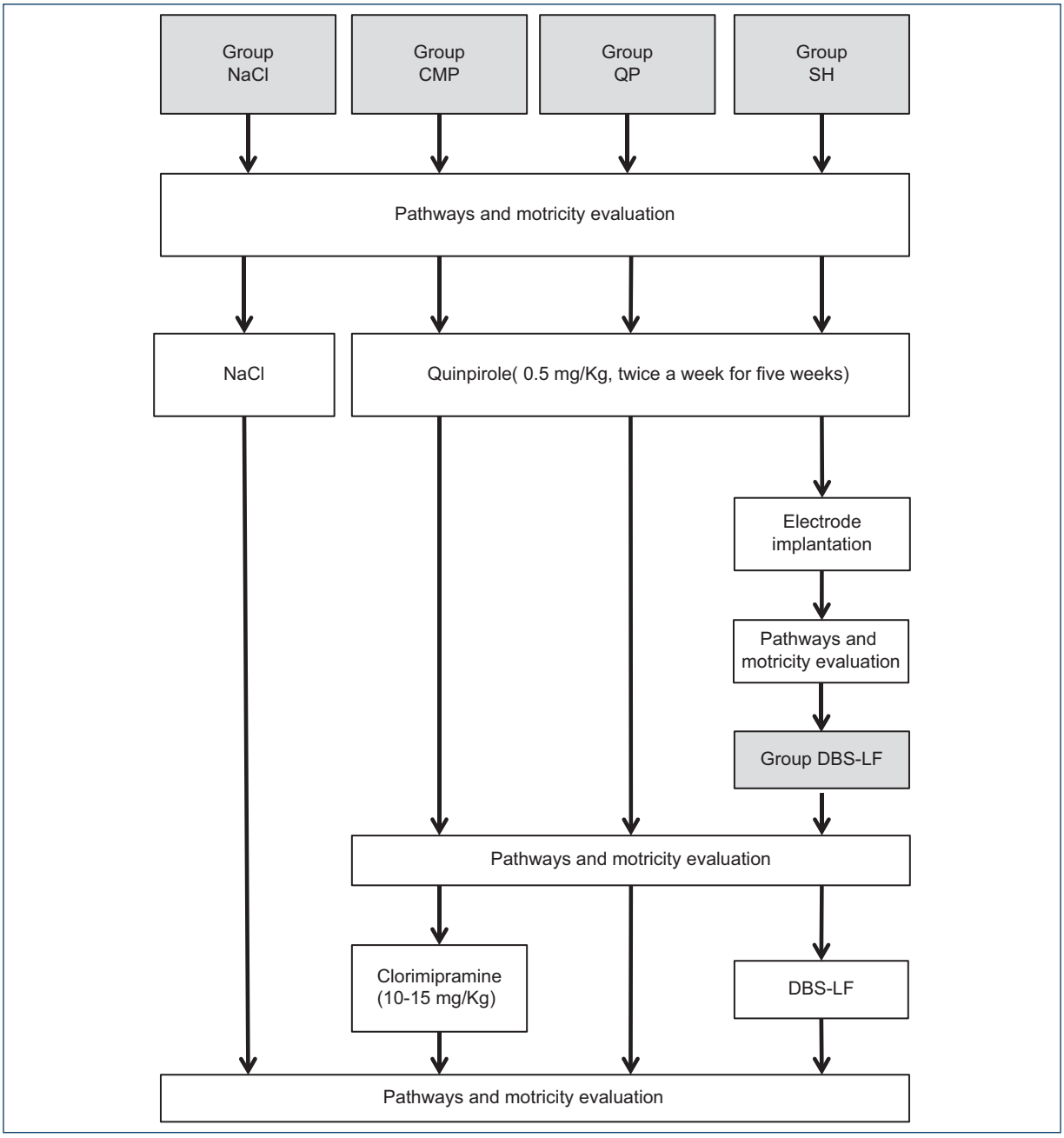

Figure 1. Treatment and procedures applied to 40 animals, each group with n = 12.

the rat was, the distance traveled in 5 min in cm was used as the main output variable. The apparatus used to evaluate this behavior was an open field plastic box with the following dimensions: 160 cm long × 160 cm wide and 60 cm high, subdivide into 25 regular quadrants.

Experimental design

Four groups were formed, the group treated with NaCl was named NaCl, the group treated with QP was named QP, and the group previously sensitized with QP and subsequently treated with CMP was named CMP. Finally, the group Sham (SH) (implanted with electrodes) in OFC without DBS was assessed in an open field. After 24 h, the same group was submitted to unilateral DBS-LF into the OFC (10.5 V, 450 micro sec, 761 W, and 6 Hz) first LF-left side and then LF-right side for 5 min each session, DBS was performed at night (Fig. 1). A stereotactic apparatus (Kopf instruments, model 960) was used for implantation.

Table 1. Differences (Δ) between NaCl versus other groups

	Δ		*p*
	NaCl		
Distance traveled in cm/5 min	QP	–390.0	0.016
	CMP	–87.5	0.582
	SH	414.5	0.020
	DBS-LF	37.0	0.829
	NaCl		
Non-visited quadrants in 5 min	QP	6.8	0.001
	CMP	2.3	0.216
	SH	–1.2	0.544
	DBS-LF	2.1	0.296
External/internal quadrants pathway	NaCl	1.96	
	QP	4.46	
	CMP	3.10	X
	SH	1.19	
	DBS-LF	1.44	

Differences (Δ) between the means of distance traveled in centimeters of the NaCl group versus each of the other groups. There is a significant difference only between the groups NaCl versus QP ($p = 0.016$) and NaCl versus SH ($p = 0.020$). In the first case, the route decreased inversely to 390 cm, in the second case, the same variable increased > 400 cm. The presence of negative sign (–) means a decrease in the traveled distance between each group. There is no significant difference between the NaCl and CMP or DBS-LF groups. There is a difference in the number of quadrants not visited only significantly in the NaCl group when compared against QP ($p = 0.001$). When establishing a ratio between the visited external quadrants over the internal ones, it is observed that for the NaCl group, there are almost twice as many visits in the former over the latter (1.96) it explains the normal behavior of the rat at the OF which is moving along the edges, whereas for the QP group, this proportion is doubled which explain PB. The DBS-LF group (1.44) is very similar to the NaCl group recovering from normal behavior. A Leven test was used to determine homoscedasticity and an ANOVA test with *post hoc* DMS and Tamhane. Statistical analysis was done with IBM SPSS Statistics V21.0 software. CMP: clomipramine, DBS-LF: deep brain stimulation low frequency, NaCl: sodium chloride, QP: quinpirole, OF: open field, SH: sham (Electrode implant without electrostimulation), X: no comparison was made for these groups.

Homemade electrodes (26 mm cannula and stainless steel cable coated with a 9 mm long insulating varnish, with the minimum resistance of 100 KΩ) were implanted. OFC coordinates were calculated from the Paxinos and Watson Atlas at 3.7 mm anterior to bregma, 2.4 mm lateral to the midline, and 4.8 mm ventral to the dura mater[21]. After 3 days of recovery, DBS was performed with a Grass Instruments S88 Dual Output Pulse Stimulator (Grass Instruments Co, West Warwick, USA).

For this study, 40 rats were divided into the following groups: Group NaCl: $n = 9$, Group QP $n = 12$, Group CMP $n = 11$, Group SH, and Group DBS-LF $n = 8$. The treatment and procedures applied to each group are shown in the following diagram.

Results

The distance traveled in 5 min in cm was used as the main output variable. In addition, the number of unvisited quadrants and the proportion of external quadrants to internal quadrants in the same period were reported (Table 1). To know the distance traveled by each rat, it was determined as the movement of locomotion when the rat moved its entire body from one quadrant to another (the four legs cross the line that divides the quadrant). For the interpretation of this movement, video recordings of each maneuver were used, the locomotion routes were marked with a permanent marker on an acetate film placed on the computer screen. Once these marked routes were established, it was possible to count how many quadrants (10 cm × 10 cm) the rat went through and interpreted as the cm traveled in 5 min.

The NaCl group obtained an average of 656.6 cm, QP group obtained an average of 266.6 cm, and CMP group obtained an average of 569 cm this group corresponds to the conventional treatment OCD (gold standard), which decreases perseverative behavior in rats. SH group obtained an average of 1071.2 cm, DBS-LF group (LF-left side) obtained an average of 693.8 cm and LF-right side obtained an average of 842.5 cm, altogether; both groups presented a greater amount of cm in comparison to the group NaCl. In the statistical analysis, we

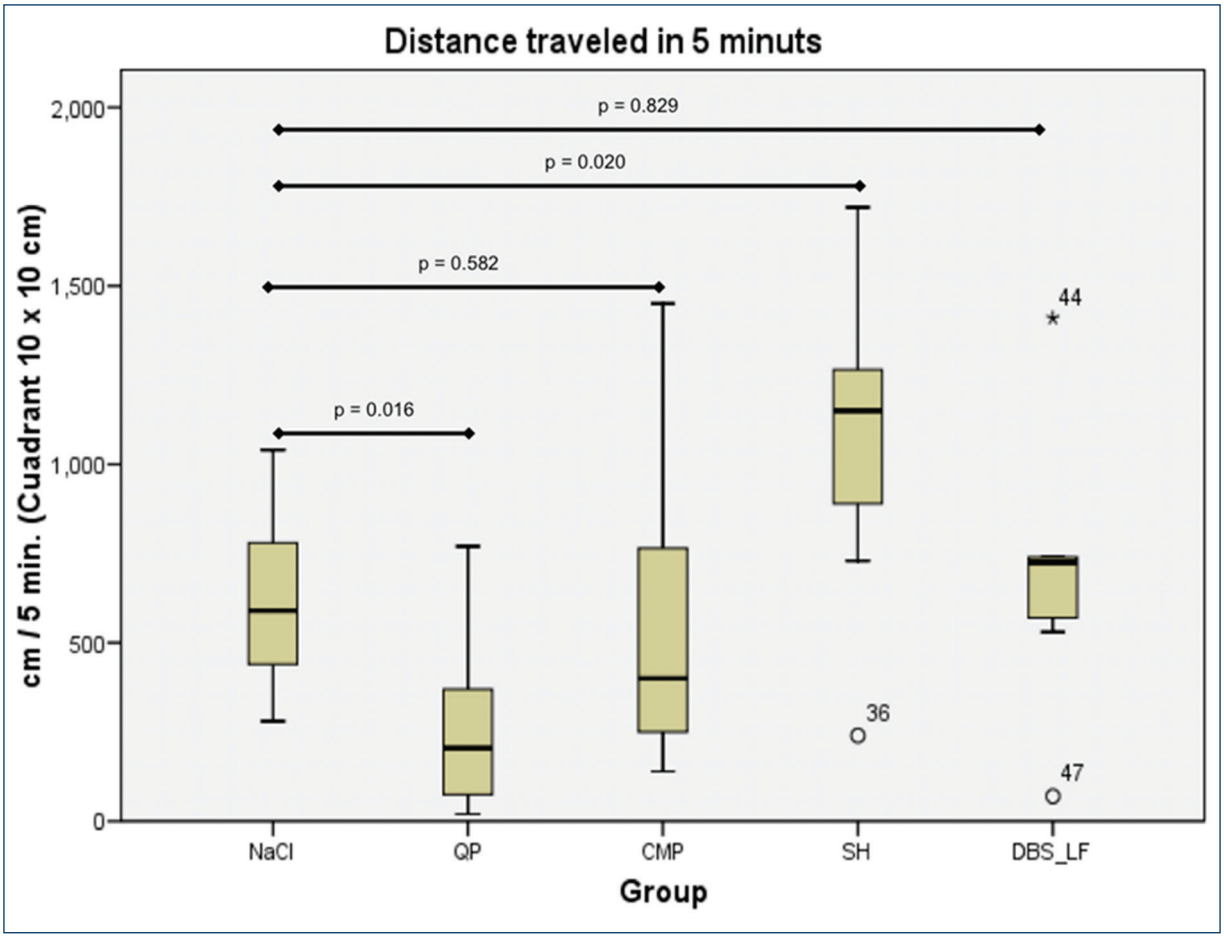

Figure 2. In this box plot, we can see the median cm traveled and their interquartile distributions and it is congruent with the Δ expressed in table 1. A decrease in motor behavior was observed for the QP group with a tendency to lead the median to low values that are interpreted as perseverative behavior, while the other groups are similar to the NaCl group, except the SH group that shows a hyperactive behavior.

can observe that when making multiple comparisons, it was found that there is no significant difference between DBS-LF-left side and DBS-LF-right side (p = 1.00).

When comparing the distance traveled (Fig. 2) in the NaCl group with the groups treated with QP (p = 0.016) and SH group (p = 0.020), a statistical difference was found, indicating the animal model was reproduced; however, when comparing NaCl group with CMP (p = 0.582), no significant differences were found, which was expected since it is the gold standard treatment. Finally, between NaCl and DBS-LF, no significant difference was found, the perseverative behavior decreased with this procedure.

Discussion

This study investigates whether DBS-LF decreases the perseverative behavior in the rat model of perseverative behavior induced by QP. The results revealed that DBS-LF reduced perseverative behavior significantly in rats. The QP-induced checking behavior is a well-established rodent model that has many features of human compulsive checking. Santoyo et al. replicated the animal model with 8 OH DPAT a 5 HT2 agonist that induces perseverative behavior in T maze apparatus and concluded that 90% of the rats decreased perseverative behavior with DBS-LF (2Hz) in OFC[22]. Some authors[23] use the number of visits to the home base rats as a variable, which consists of determining how many times the rodent visits a place in the open field, a site predetermined by the researcher, i.e., a housing cage. It can also be measured by how often rodents come into contact with objects placed at different points of the open field. The home base model can explain better the perseverative behavior naturally, but it also promotes memorization and reinforcement. In our study, although the rat is not in a natural environment, we eliminate home base and the visit objects to avoid memory bias

or reinforcement. However, for the apparatus that we used the rats could at first seek an outlet to escape, with a tendency to take refuge in the edges, unlike the rats treated with QP, NaCl group has a more uniform distribution of locomotion compared to QP group, which have a tendency to stay longer in certain places performing grooming, yawning, and licking.

The weaknesses of this study could be focused on not applying DBS-LF bilaterally at the same time and not having brain histology available to corroborate misplaced electrodes. Previous studies showed that bilateral low frequency can avoid perseverative behavior in an 8 OH-DPAT model[14,22]. Seeman et al. concluded that the administration of ionotropic glutamate receptor antagonists such as phencyclidine, ketamine, and dizocilpine binds to dopamine D2 receptors, which may condition competitive agonist with QP at D2/D3 receptors during anesthetic procedure, thus modifying the behavior of the rat[24].

Gök et al. reported that the administration of the metabotropic antagonist 5 receptors of glutamate named 2-methyl-6-phenylethynyl-pyridine (1 or 5 mg kg–1, i.p.) before each QP injection inhibited significantly the increase of locomotion and compulsive control behavior of rats[25].

QP or a 5-HTA2 agonist such as 8-OH-DPAT can induce perseverative behavior, respectively, in open field and T-maze test by acting on different parts of the underlying motivation safety circuit in OCD. QP acts directly through dopaminergic pathways, motivating a checking activity, and 8-OH-DPAT perpetuates motivational activity by inhibition of negative feedback pathway.

Perseveration of routes and hyperactivity observed in the SH group could be the mechanic effect of the electrode associated to QP[26]. Comparing the NaCl and SH groups, we found that there was a significant difference in the distance traveled in 5 min between these groups (p = 0.020). In this study, the electrode implantation itself is capable to produce a change in the behavior of the rat; this change may be due to the microinjury effect[27-29] and QP pharmacokinetics (hyperkinetic phase). Hartesveldt proposes that QP has the capacity to induce suppressive effects on locomotion and the ability to cause an increase in motor activity[30].

According to the results of this study, unilateral DBS-LF can reduce the perseverative behavior in a QP model. On the other hand, Sturm et al. have published unilateral DBS in accumbens nucleus can reduce OCD symptoms in patients[18]. Specific clinical trials should be considered to get enough information on the safety and effectiveness of unilateral DBS in human being.

Conclusions

Chronic QP (0.5 mg/kg subcutaneous twice weekly for 5 weeks) produces perseverative behavior in Wistar rats; this behavior is manifested by a decrease in locomotion and an increase of the visits of certain quadrants of the open field.

Unilateral DBS-LF decreases perseverative behavior in Wistar rats induced by QP animal model in an open field test.

Dopamine plays a role in the development of perseverative behavior in the rat and can be modulated by electrostimulation at low frequency (6 Hz).

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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