A decrease in gamma-synuclein expression within the nucleus accumbens increases cocaine intravenous self-administration in the rat

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ABSTRACT

Except as a marker of cancer progression, gamma-synuclein (GSyn) had received little attention. Recent data showed however that GSyn modulates cocaine-induced locomotor effects, suggesting that it could also play a role in cocaine reinforcing effects. In the rat, siRNAs targeting GSyn expression were injected in the nucleus accumbens and cocaine reinforcing effects were evaluated by means of intravenous self-administration. A dose-response curve was followed by procedures of progressive ratio, extinction, cocaine- and cue-induced reinstatements. Decrease of GSyn expression increased self-administration over a large range of doses. This effect was associated with an increase in cocaine-induced reinstatement. The present data reveal that GSyn exert a specific negative control on cocaine-induced reinforcing and incentive effects.

Keywords Cocaine, gamma-synuclein, incentive, intravenous self-administration, rat, siRNA.

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Synucleins are three soluble proteins expressed in neural tissue (Surguchov 2008): alpha- (Asyn), beta- and gamma-synuclein (GSyn). Consistent with its role in dopamine homeostasis (Xu et al. 2002), ASyn has been unsurprisingly associated with drug use-related disorders (Liang et al. 2003; Mash et al. 2003; Bonsch et al. 2004). GSyn is distinct from the two other family members in terms of structure and cell localization and has been mainly characterized as a marker of cancer progression (Surguchov 2008). Recent data, however, revealed that GSyn can modulate dopaminergic activity (Moszczyńska et al. 2007) and cocaine-induced locomotor effects (Boyer & Dreyer 2008). We therefore hypothesized that GSyn might also modulate cocaine reinforcing effects and therefore cocaine use.

In the rat, lentiviruses driving the expression of small interfering RNAs against GSyn mRNA (LV-GSyn-siRNAs) were administered in the nucleus accumbens, a key structure mediating cocaine-induced reinforcing effects (Kalivas, Volkow & Seamans 2005). Cocaine use was evaluated using intravenous self-administration (SA). siRNAs targeted against GSyn mRNA or Green Fluorescent Protein mRNA (LV-GFP, control) were produced (Bahi et al. 2004a; Bahi et al. 2004b; Boyer & Dreyer 2008) and administered using a classical stereotaxic approach. In the same time, rats were equipped with an intravenous catheter. Seven days later, rats were trained to self-administer cocaine at a dose of 0.8 mg/kg/infusion for 12 days (see Appendix S1 in the Supporting Information for details on the experimental SA set-up and procedures). During the acquisition phase, stability in drug intake (Fig. 1a) and preference for the active device (a hole in which nose-poking induces cocaine delivery), against the inactive one [hole, F(1,15) = 35.2, P < 0.0001] (data not shown), attested to the fact that cocaine was self-administered. During this acquisition period, LV-GSyn-siRNAs did not alter cocaine intake, total responding or discrimination between active and inactive devices.

A dose-response curve was then performed. Both the dose-response functions for cocaine intake [dose, F(4,64) = 22.06, P < 0.0001; group effect, F(1,16) = 5.618, P < 0.05, group × dose, F(4,64) = 1.721,
P = 0.15] and active responses [dose, F(4,64) = 13.16, P < 0.0001; group, F(1,16) = 4.03, P < 0.05, groupx-dose, F(4,64) = 0.69, P = 0.60] were shifted upward in LV-GSyn-siRNAs rats (Fig. 1b & c). Whatever the cocaine dose, inactive responses were similar in the two groups, suggesting that LV-GSyn-siRNAs treatment produced a specific increase in cocaine reinforcing effects.

To fully characterize the psychopharmacological consequences of LV-GSyn-siRNAs, we then performed: (1) a progressive ratio schedule evaluating motivation for the drug (Richardson & Roberts 1996); (2) an extinction procedure, during which the drug is withdrawn and responding is without scheduled consequences (Extinction is considered as a new learning resulting in an active inhibitory control over the learned operant behavior (Bouton 2004; Woods & Bouton 2007). The extinction procedure allows then measuring the ability to gain control over the behavioral response previously leading to cocaine infusions); and (3) cocaine- and cue-induced reinstatements (Shaham & Hope 2005). Small amounts of the abused drug, and drug-associated contextual cues, are factors known to possess incentive effects which can precipitate drug-seeking (Shaham & Hope 2005).

Whatever the dose of cocaine tested, LV-GSyn-siRNAs treatment highly tended to increase motivation for cocaine as measured by the breakpoint (BP; last ratio completed) in the PR schedule (Fig. 2a) [F(1,15) = 1.85, P = 0.18]. On the contrary, the two groups did not differ for responding on both active and inactive devices during extinction (Fig. 2b).

Contingent presentations of the cocaine-associated cue light produced a significant reinstatement of the extinguished SA behavior (Fig. 2c and Appendix S2 in the Supporting Information) as attested by a higher number of responses in the active than in the inactive hole [hole, F(1,15) = 12.09, P < 0.005]. This effect was, however, similar in both experimental groups.

Cocaine produced a dose-dependent [dose, F(3,45) = 9.32, P < 0.0001] and specific [hole, F(1,15) = 10.16, P < 0.01; holex-dose, F(3,45) = 7.08, P < 0.0005] reinstatement of the SA behavior (Fig. 2e). Indeed, responding in the inactive hole was not altered by cocaine and responding in both holes was not altered by vehicle infusions in the same experimental conditions (Fig. 2d, and Appendix S2 in the Supporting Information). Interestingly, cocaine-induced reinstatement was higher in LV-GSyn-siRNAs rats as compared with LV-GFP rats [groupx-dose, F(3,45) = 2.92, P < 0.05] and this effect was specific of the active hole [groupx-dosex-hole, F(3,45) = 2.51, P < 0.05].
Rats were killed 24 hours after the last SA session and nucleus accumbens sampled. GSyn transcripts were quantified by quantitative real-time PCR (qPCR). GSyn and Asyn proteins were quantified by Western blots (see details in Appendix S1 in the Supporting Information). Animals injected with LV-GSyn-siRNAs displayed a 50% decrease in GSyn mRNA (Fig. S1A) and GSyn protein levels (Fig. S1B). The observed behavioral effects of LV-GSyn-siRNAs administration were not due to compensation in ASyn expression. ASyn protein levels in the two experimental groups (Fig. S1C) were comparable confirming previous data (Boyer & Dreyer 2008).

Recent data suggested that GSyn might play a role in cocaine reinforcing effects (Boyer & Dreyer 2008). By using siRNAs, we decreased GSyn expression in the nucleus accumbens, a brain structure thought to be a key actor for cocaine use, abuse and addiction (Kalivas et al. 2005). This manipulation increased cocaine SA. This increase was specifically associated with increased cocaine-induced incentive effects and a high trend to an increased motivation. It did not alter behavioral disinhibition as measured in an extinction procedure and cue-induced reinstatement. GSyn may exert a specific negative control on cocaine reinforcing and incentive effects in the nucleus accumbens.

In the present work, silencing of GSyn was shown to increase cocaine reinforcing and incentive effects, while
the same manipulation was previously shown to decrease cocaine-induced locomotion (15 mg/kg) (Boyer & Dreyer 2008). Further investigations are needed to explain what appears as a discrepancy. Since only one dose of cocaine was tested on locomotor activity, the decreased response could indeed reflect an increased cocaine efficacy expressed by increased stereotyped behaviors.

Neurobiological mechanisms underlying the modulation of cocaine reinforcing effects by GSyn have now to be investigated. Modulation of the DAT function might be a target of interest as GSyn has been shown in vitro to directly interact with the DAT and disrupt the ASyn-DAT interaction (Moszczynska et al. 2007). This mechanism fits with the effect of the combined manipulations of the DAT and GSyn levels on cocaine locomotor effects (Boyer & Dreyer 2008).

Acknowledgements
This work was supported by the following grants: ANR-Addiction (2005) and EU-STREP-PheCOMP (FP6) (PVP), MILDT/INCa/Inserm (2008) (VDG), Swiss National Foundation grants 3100-059350 and 3100AO-100686 (JLD). The authors are very grateful to Mr. V. Chandrasekar and Mrs. C. Deforel-Poncet for their skilful assistance. The authors report no financial conflict of interest.

Authors contribution
VDG and JLD designed the study. FB and EB performed the experiments. VDG supervised the study and wrote the article. VDG and PB analyzed the data. VDG, JLD and PVP provided funds. All authors have critically reviewed content and approved final version submitted for publication.

References

Supporting Information
Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supplementary Methods
Appendix S2 Supplementary Results
Figure S1 Gamma- and alpha-synuclein expression in the nucleus accumbens of rats administered with LV-GSyrn-siRNAs (grey bars) and rats administered with LV-GFP (controls, white bars).

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