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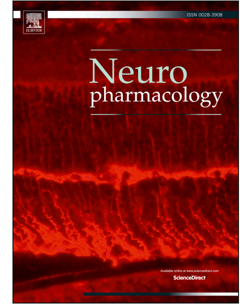

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Activation of D2 autoreceptors alters cocaine-induced locomotion and slows down local field oscillations in the rat ventral tegmental area

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Keywords : stereotypies; local field potential; ventral tegmental area; cocaine; quinpirole

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ABSTRACT

Psychoactive substances affecting the dopaminergic system induce locomotor activation and, in high doses, stereotypies. Network mechanisms underlying the shift from an active goal-directed behavior to a "seemingly purposeless" stereotypic locomotion remain unclear. In the present study we sought to determine the relationships between the behavioral effects of dopaminergic drugs and their effects on local field potentials (LFPs), which were telemetrically recorded within the ventral tegmental area (VTA) of freely moving rats. We used the D2/D3 agonist quinpirole in a low, autoreceptor-selective (0.1 mg/kg, i.p.) and in a high (0.5 mg/kg, i.p.) dose, and a moderate dose of cocaine (10 mg/kg, i.p.). In the control group, power spectrum analysis revealed a prominent peak of LFP power in the theta frequency range during active exploration. Cocaine alone stimulated locomotion, but had no significant effect on the peak of the LFP power. In contrast, co-administration of low dose quinpirole with cocaine markedly altered the pattern of locomotion, from goal-directed exploratory behavior to recurrent motion resembling locomotor stereotypy. This behavioral effect was accompanied by a shift of the dominant theta power toward a significantly lower (by ~15%) frequency. High dose quinpirole also provoked an increased locomotor activity with signs of behavioral stereotypies, and also induced a shift of the dominant oscillation frequency toward the lower range. These results demonstrate a correlation between the LFP oscillation frequency within the VTA and a qualitative aspect of locomotor behavior, perhaps because a variable level of coherence of this region with its input or output areas.

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Introduction

In any brain area, the simultaneous activity of thousands of cells generates rhythms which rapidly fluctuate during wakefulness (see (Buzsaki et al., 2012) for a review). A central question in Neuroscience is how the brain coordinates the activity of various cortical and subcortical networks to produce behavioral output. In this respect dopaminergic (DAergic) psychostimulants can be used as a research tool to decompose complex exploratory behavior into simpler behavioral units (Berridge, 2012; Teitelbaum et al., 1990). These simpler behaviors can be analyzed further to discover the brain mechanisms that control them.

The primary targets for psychoactive drugs are DAergic projections from the ventral tegmental area (VTA) to the forebrain regions. DAergic and GABAergic neurons composing the VTA network (Creed et al., 2014; Nair-Roberts et al., 2008; Oades and Halliday, 1987) are arranged in “subnetworks” with specific inputs and outputs (Beier et al., 2015; Lammel et al., 2008; Lammel et al., 2011; Watabe-Uchida et al., 2012). How these interconnected subnetworks operate over time in natural conditions is not clear, but the VTA in general is concerned with motor activity, motivation, reward and salience (Bromberg-Martin et al., 2010; Schultz, 2007) – components of the goal-directed behavior. At the same time, recent research clearly demonstrates that goal-directed behavior is preconditioned by the phase coupling of the local field potential (LFP) oscillations across various brain areas, including the prefrontal cortex, the VTA, and the hippocampus (Fujisawa and Buzsaki, 2011; Pezzulo et al., 2014).

In the present study we used the D2/D3 agonist quinpirole and the triple reuptake inhibitor cocaine to affect rats’ behavior, particularly to induce stereotypic locomotion, in combination with telemetry to measure the LFP oscillations within the VTA. The goal was to test the hypothesis that alterations in locomotor behavior are due to changes in the activity of the VTA network.

We focused on the LFP frequency band of 4-10 Hz. This rhythm is commonly referred to as *hippocampal theta oscillations* (Buzsaki, 2002), but it can be recorded in many areas throughout the brain (Stewart and Fox, 1990). Interestingly, a recent study demonstrated a clear correlation between theta rhythms in the VTA and in the hippocampus (Orzel-Gryglewska et al., 2014). In awake animals, theta oscillations are associated with active motor behavior such as walking or exploratory sniffing, and play a critical role in cognitive processes such as spatial learning, navigation, and attention (Buzsaki, 2002, 2005; Seidenbecher et al., 2003; Siapas et al., 2005).

Our results strongly suggest a link between a shift in the peak frequency of the LFP oscillations recorded within the VTA network and drug-induced alterations of locomotor behavior.

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Materials and Methods

Subjects. 36 adult male Wistar rats, weighing 250-300 grams, were implanted with a microelectrode array consisting of 8 platinum-iridium electrodes and designed so as to span the whole extent of the VTA (see details below and in Koulchitsky et al, 2012). All animals were housed individually and maintained on a 12-h light : 12-h dark cycle. Water and food were available ad lib. All animal care and handling was conducted in accordance with the guidelines stated in the Handbook for the Use of Animals in Neuroscience Research (Society for Neuroscience, 1991). All procedures were also carried out in accordance with guidelines of the European Communities Council Directive of 24 November 1986 (86 �609 �EE) and were approved by the Ethics Committee for Animal Use of the University of Liege (protocol 917).

Surgical and histological procedures were as described in detail elsewhere (Koulchitsky et al., 2012). Briefly, animals were placed in a stereotaxic apparatus (Model 902, Kopf). General anesthesia was induced and maintained using chloral hydrate (400 mg �kg, i.p.). Soft tissues of the skull were anesthetized using a 0.5% lidocaine hydrochloride. Body temperature was maintained at 36–37°C by means of a heating pad. The exposed bone was cleaned with saline and a 3% hydrogen peroxide solution. The area of entry was defined according to stereotaxic coordinates (Paxinos and Watson, 2007). A small part of the skull between the lambda and the bregma was removed above the implantation point using a dental burr (Microtorque II control box, Tech 2000 Handpiece, Ram Products Inc., Encino, CA, USA). A microelectrode array (custom made MEA, 8 recording and one reference electrodes, Alpha-Omega, Jerusalem, Israel) was lowered into the VTA through the opening (5.8-6.8 mm posterior to the bregma, 0.6-0.9 mm lateral to the midline, and 7.5–8.5 mm under the cortical surface) using a micromanipulator, and fixed to the skull using anchoring stainless steel screws with Z100™ and dental restorative Adper™ Scotchbond™ multi-purpose adhesive (3M ESPE Dental Products, St. Paul, MN, USA).

At the end of the experimental series, the rats were deeply anesthetized with pentobarbital (200 mg/kg). The recording site was then marked by an electrolytic lesion of the brain tissue through a previously implanted electrodes (1.0 mA cathodal current for 6 s), and animals were perfused intracardially with saline followed by a 4% formaldehyde saline solution. The brains were then removed and stored for histological examination (Koulchitsky et al., 2012).

Electrophysiology. Local field potentials were recorded using a newly developed wireless recording system (W8 system, Multi Channel Systems GmbH, Reutlingen, Germany). Microelectrode arrays were produced by Alpha Omega GmbH (Israel). Recording electrodes were made of platinum-iridium with Parylene C coating (1 MΩ impedance at pre-characterization). The reference electrode was made of the same material (25 kΩ impedance at pre-characterization) and was positioned on the skull nearby. Sampling rate was 20 kHz with no band-pass on-line filtering. In this study, only signals between 1 and 300 Hz were analyzed. Action potentials from individual neurons were not examined.

Experimental procedure. The transmitter was connected to the pre-implanted electrodes 5 min before the experiment. Each rat was then placed into the center of a 43.2×43.2×30 cm (width × length × height) Plexiglas chamber. Locomotor activity was monitored using a MED-OFA Activity Monitor (MED Associates Inc, St. Albans, VT, USA). Acquisition frequency was 20 Hz. After 45 min of baseline recording, each animal was gently taken from the chamber for an injection. Immediately after the injection, the rat was returned to the chamber, and the recording continued for another 60 min.

All drugs were injected intraperitoneally. Each animal was used for the injection of saline or one of the following: quinpirole (0.1 mg/kg; referred to as low-dose) + cocaine (10 mg/kg), cocaine (10 mg/kg), quinpirole (0.5 mg/kg; referred to as high-dose). Co-administration of low-dose quinpirole and cocaine was done in a 15 min interval. This interval was chosen because, according to our previous experience, the behavioral and electrophysiological effect of this dose of quinpirole reaches

its maximum at this time (Koulchitsky et al., 2012). Also, because preliminary experiments demonstrated that low-dose quinpirole alone strongly suppresses locomotion, this drug regimen was not investigated in this study.

Data analysis. Electrophysiological and behavioral data were exported to Matlab *.mat files and analyzed using Matlab software (Version 7.7.0.471). Locomotor behavior and, in some groups, path stereotypies, were most clearly expressed between 10 and 30 min after the injections of dopaminergic drugs (after the cocaine injection in the case of low dose quinpirole + cocaine). Therefore, spectral power analysis of the LFPs was performed during that period, and within the stereotypic episodes in the groups in which they occurred. ~~LFP frequency spectra were built using the MATLAB FieldTrip toolbox (Oostenveld et al., 2011)~~. LFP frequency spectra were built using the multitaper method based on discrete prolate spheroidal sequences (Slepian sequences) as tapers (MATLAB FieldTrip toolbox; (Oostenveld et al., 2011)).

Animals in different groups have different level of locomotor activity, and different maximal speed of locomotion. Voluntary locomotion by itself is associated with changes in the firing rate of a majority of VTA DA neurons (Koulchitsky et al., 2012), and with modifications of the frequency of brain oscillations (Oddie and Bland, 1998; Orzel-Gryglewska et al., 2014; Vanderwolf, 1969). This created a problem for the direct comparison of LFP frequency spectra across different groups. To overcome this, the following approach was chosen. From the behavioral data of each electrophysiological experiment we extracted locomotor bouts, 10 s each, with a similar velocity pattern across all groups (Figure S1). Maximal velocity of the selected bouts varied by no more than ±25%. The general procedure used for the extraction and analysis of corresponding electrophysiological data is described schematically in the supplementary Figure S1. Spectral analysis of each LFP segment, corresponding to the locomotor bouts, was performed as described above. From each power frequency spectrum we identified a frequency at which the power reached a maximal value. These values were used for statistical comparison between the groups.

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Behavioral data were analyzed using Matlab (version 8.3.0.532, R2014a). The position data was smoothed using a moving average filter of width 11, corresponding to a 0.5 s window. Speed was defined as the Euclidean distance of the position at two consecutive time points divided by the sampling time, and resting as zero-speed. A complete circle was defined as a movement around one of the 256 (16 x 16) grid points of MED-OFA Activity Monitor with a minimum radius of 2.5 cm and at most 18° backward motion. Temporally overlapping circles were discarded.

Drugs and chemicals. Cocaine hydrochloride for i.p. injection was obtained from Fagron (Waregem, Belgium). Heparin was from LEO Pharma (Lier, Belgium). Quinpirole was obtained from Tocris Bioscience (Bristol, United Kingdom).

Statistical analysis. Electrophysiological data are presented as means ± SEM. Comparison of these data was performed by either analysis of variance (analysis over the whole 20 minute period) or a hierarchical analysis of variance (ANOVA) (analysis of locomotor bouts). In the latter analysis, we collected several locomotor bouts for each rat. This gave us several corresponding LFP segments, and thus several values of frequency at which oscillation power reached its maximum. Frequency values were nested within the rats, which were in turn nested within the treatment groups. The groups were treated as a between-subject factor. Because behavioral data were not normally distributed, we analyzed them using non parametric statistics (Kruskal-Wallis ANOVA followed by two-tailed Wilcoxon rank sum tests, the level of significance of the Kruskal-Wallis test is indicated in the legend of the relevant figures). Statistical significance was set at $p < 0.05$. Behavioral data are represented graphically as box plot, with the box covering 25th to 75th percentiles, and the whiskers extending to the highest and lowest value within 1.5 times the interquartile range. A more classical representation (mean ± SEM) is also provided in the figure.

Results

Behavioral results

We compared the locomotor activity of rats in the baseline condition and after the intraperitoneal injections of saline (N = 15 rats), 10 mg/kg cocaine (N = 6 rats), low-dose (0.1 mg/kg) quinpirole + 10 mg/kg cocaine (N = 8 rats), or high-dose (0.5 mg/kg) quinpirole (N = 7 rats). Behavior of the animals was not different during baseline and after the injection of saline (data not shown).

As expected, low-dose quinpirole by itself strongly decreased locomotor behavior. However, after the subsequent injection of cocaine we observed a marked increase of locomotor activity, i.e. time spent moving (Figure 1 A), as compared to the saline group (pairwise two-tailed Wilcoxon rank sum test ($p < 10^{-5}$, rank sum = 120). Injections of cocaine alone and of high-dose quinpirole also increased the time spent moving, as compared to the control conditions. Pairwise two-tailed Wilcoxon rank sum tests confirmed the significant difference in the cases of the control *versus* cocaine ($p = 0.0015$, rank sum = 122) and control *versus* low-dose quinpirole + cocaine ($p < 10^{-5}$, rank sum = 120).

No significant difference was found in the other pairs, except for high-dose quinpirole animals who spent less time moving than animals of the low-dose quinpirole + cocaine group ($p < 0.05$, rank sum = 86). Consistent results were obtained in terms of the distance travelled (Figure 1 B). All drug-treated groups travelled a longer distance than the saline group (cocaine: $p = 0.0011$, rank sum = 126; low-dose quinpirole + cocaine: $p < 10^{-5}$, rank sum = 121; high-dose quinpirole: $p = 0.0015$, rank sum = 130). Also the low-dose quinpirole + cocaine group travelled a longer distance than the high-dose quinpirole ($p = 0.0289$, rank sum = 83).

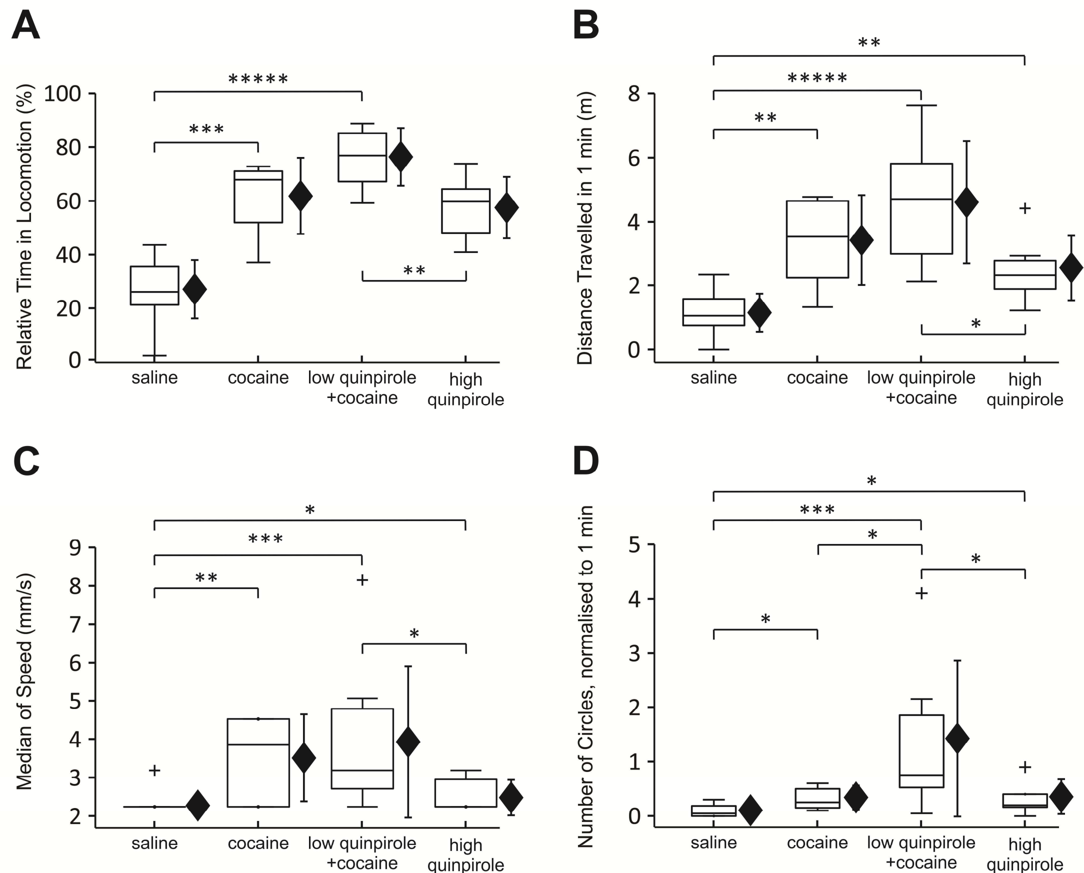

Figure 1. Behavioral effects of various drug regimens. A. Percentage of time during which animals from the different groups moved (Kruskal-Wallis test, $k = 27.01$; $p < 10^{-5}$; $df = 3$). Data are represented graphically as box plot, with the box covering 25th to 75th percentiles, and the whiskers extending to the highest and lowest value within 1.5 times the interquartile range. A representation as mean (black diamonds) ± SEM is also provided on the right side of each plot. **B.** Average distance travelled per minute by the animals from the different groups (Kruskal-Wallis test, $k = 22.86$; $p < 10^{-4}$; $df = 3$). **C.** Median speed of animals from the different groups (Kruskal-Wallis test, $k = 18.45$; $p < 10^{-3}$; $df = 3$). **D.** Number of complete circles (see methods for their definition) made by the animals from the different groups (Kruskal-Wallis test, $k = 16.37$; $p < 10^{-3}$; $df = 3$). The degree of significance is represented as * $p < 0.05$, ** $p < 10^{-2}$, *** $p < 10^{-3}$, ***** $p < 10^{-5}$.

In terms of speed (Figure 1 C), the control group was slower than all other groups (cocaine: p = 0.0018, rank sum = 128; low-dose quinpirole + cocaine: p = 0.00029, rank sum = 128; high-dose quinpirole: p < 0.05, rank sum = 144.5), as was high-dose quinpirole *versus* low-dose quinpirole + cocaine, and cocaine alone (p < 0.05, rank sum = 82; p =0.04, rank sum = 53.5, respectively).

Interestingly, we observed that two groups – cocaine and low-dose quinpirole + cocaine – spent less time at slow speed (< 5mm/s) and more time at medium speed (between 5 and 15 mm/s) than the other two groups, while the time at higher speed (> 15 mm/s) was comparable across all groups (Supplementary Figure S2).

We next analyzed in detail specific aspects of the locomotor behavior during the various experimental conditions. After the injection of the low-dose quinpirole + cocaine animals expressed clear signs of locomotor stereotypy – rhythmic repetition of the locomotor paths (see Supplementary Video 1)– known to be induced by some dopamine receptors agonists (for review see (Eilam et al., 2006; Mason, 1991)) (for examples see inset in Figure 2 C). This was reflected in a significantly larger number of circles in the open field arena completed by the animals from low-quinpirole + cocaine group as compared to the rats in the control (p = 0.0003, rank sum = 128.5), and cocaine alone (p = 0.046, rank sum = 29.5) and high dose of quinpirole (p = 0.034, rank sum = 82) groups. Animals in the cocaine and high dose of quinpirole groups also completed more circles than those of the control group (p = 0.0128, rank sum = 134 and p = 0.0346, rank sum = 143, respectively) (Figure 1 D).

In the high-dose quinpirole group, locomotor activation was accompanied by characteristic signs of focused stereotypy, mainly perseverative sniffing (see Supplementary Video 2).

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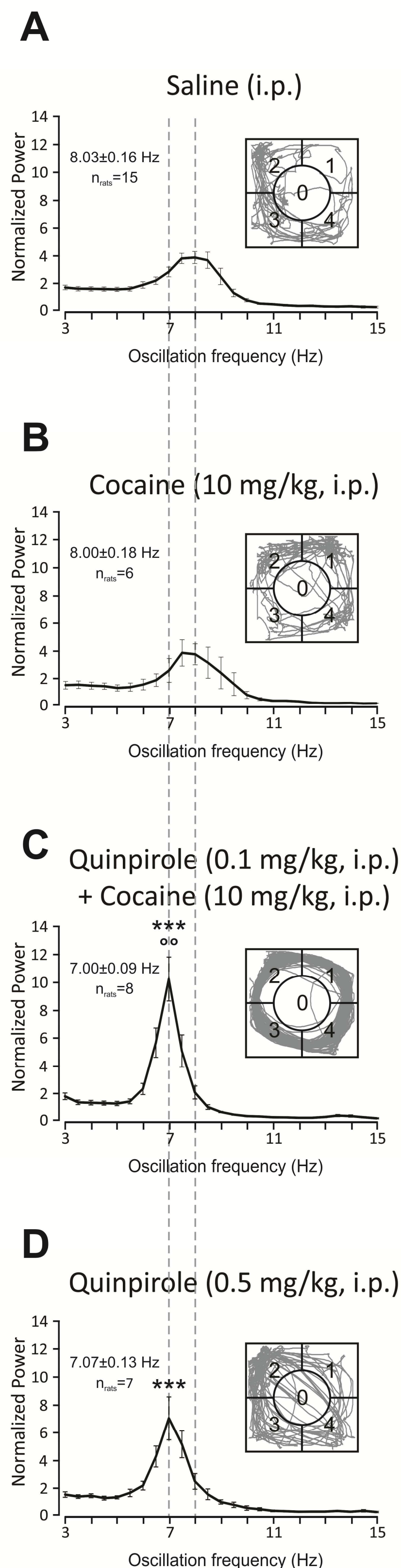
Analysis of electrophysiological data



We first analyzed the 20 min periods of LFP recordings corresponding to the active locomotion (see Methods). Power spectrum analysis revealed a prominent peak of power in the theta range in all groups (Figure 2 A to D). In the low-dose quinpirole + cocaine and the high-dose quinpirole groups the peaks occurred at significantly lower frequencies than in the saline and cocaine groups (see Table 1 for precise values). The peak frequency in the cocaine alone group was not significantly different from the one of the control group.

In addition, the maximal power of theta oscillations in the low-dose quinpirole + cocaine group, but not the high dose quinpirole group, was significantly higher than in the saline and cocaine alone groups.

Figure 2. Electrophysiological effects of various drug regimens. A to D. Averaged power spectral densities of the LFPs recorded from the 10th to the 30th min after the injection in the different groups. Vertical dashed lines mark the peaks of frequency power in the saline and low-dose quinpirole + cocaine groups, respectively. Squared insets at the right side of each graph are schemes of the open-field arena with a corresponding example of the locomotor trajectories. The degree of significance versus the saline group is represented as °°$p < 10^{-2}$ (for the power), ***$p < 10^{-3}$ (for the frequency).

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Table 1. Maximal power (means of normalized values ± SEM), and corresponding frequencies (means ± SEM) of the LFP oscillation recorded in the different groups at the period from the 10th to the 30th min after the injection. For the low-dose quinpirole + cocaine group the period is taken after the second injection.

Group	Power (normalized units)	Frequency (Hz)	N_{rats}
Saline (i.p.)	5.29±0.46	8.03±0.16	15
Cocaine (10 mg/kg, i.p)	5.48±1.33	8.00±0.18	6
Low-dose quinpirole (0.1 mg/kg, i.p.) + Cocaine (10 mg/kg, i.p.)	10.41±1.50**/°	7.00±0.09***/°°	7
High-dose quinpirole (0.5 mg/kg, i.p.)	7.42±1.47	7.07±0.13***/°°	7

ANOVA test ($F_{frequency}$ (3,32) = 12.329, $p < 10^{-4}$; F_{power} (3,32) = 5.128, $p < 10^{-2}$), followed by Tukey HSD post-hoc (**$p < 10^{-2}$, *** $p < 10^{-3}$ for comparison to saline group; ° $p < 0.05$, °° $p < 10^{-2}$ for comparison to cocaine group).

To eliminate the biases due to changes in the firing rates of some VTA neurons induced by locomotion (Koulchitsky et al., 2012), and the effects of differences in the speed of locomotion (Slawinska and Kasicki, 1998), we performed an additional analysis. As described in detail in the methods and in supplementary Figure S1, we extracted locomotor bouts, 10 s each, with similar patterns across groups, and took the corresponding LFP segments for frequency analysis and comparison. Some rats from the low-dose quinpirole + cocaine did express minimal locomotor activity in the period between the quinpirole and cocaine injections (at a time when the animals already had a reduced global motor activity). This allowed us to extract some locomotor bouts and we used corresponding LFP segments in this session of the analysis. As can be seen from Table 2 and on Figure 3, power spectrum analysis of the segments revealed similar differences between the frequency power peaks in the theta range across the groups (see also Table 1 and Figure 2 for comparison). Dominant frequency values from the low-dose quinpirole + cocaine, high-dose

quinpirole, or low-dose quinpirole groups were all different from the ones of the saline and cocaine groups. Values in the two latter groups were similar. Interestingly, analysis of the segments extracted from the period between the low-dose quinpirole and the cocaine injections demonstrated that by itself quinpirole at this dose provokes a significantly smaller shift of the peak theta power toward the lower frequency.

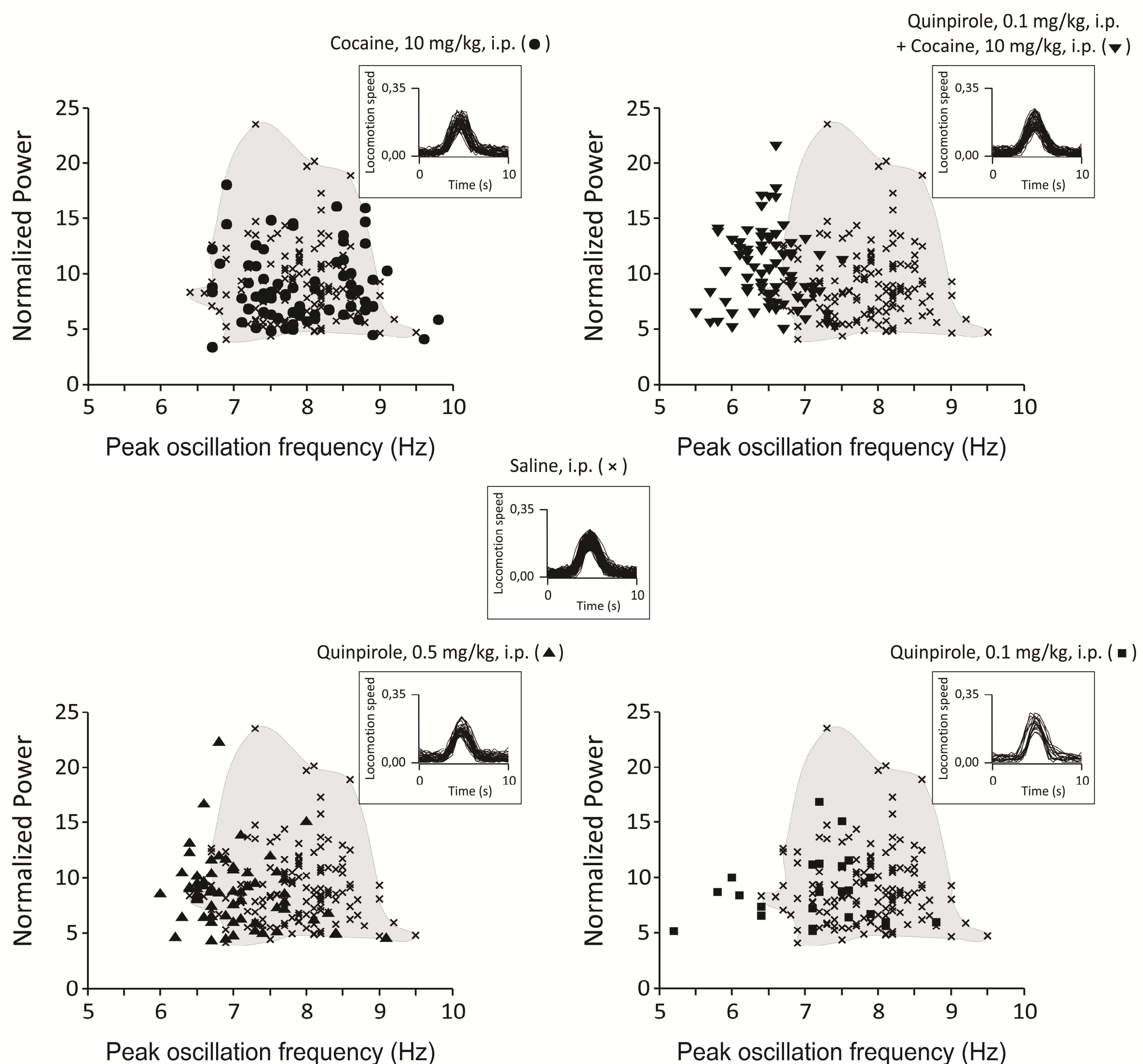

Figure 3. Local field potential characteristics during the locomotor bouts in the different groups. Scatter plots depicting the frequency of the peak power (X axis) and the peak power normalized to the average power (Y axis), calculated for the 10 s LFP segments. In each panel, individual values

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during various drug regimens are compared to the values of the saline group (crosses, represented in each panel for comparative purposes). In addition, the area occupied by the saline values is outlined by the dashed line and filled in gray. Insets show the corresponding 10 s locomotor bouts. The number of segments used and the statistical values are indicated in Table 2.

Noticeably, according to this analysis, the maximal power of theta oscillations in the low-dose quinpirole + cocaine and cocaine alone groups was not different from that of the saline group and were similar to each other. This result contrasts to the analysis over the whole of 20 min periods, suggesting that the increase in theta LFP power observed in the first analysis is due to the general increase in locomotor activity (see Discussion). In addition, in the segments extracted in the low- and high-dose quinpirole groups, the maximal power was significantly lower than in saline and low-quinpirole + cocaine, but not different from that of the cocaine group (Table 2).

Table 2. Maximal power (means of normalized values ± SEM), and corresponding frequencies (means ± SEM) of the LFP oscillation in the different groups, calculated for the LFP segments corresponding to the 10 s locomotor bouts

Group	Power (normalized units)	Frequency (Hz)	N_{rats} ($N_{segments}$)
Saline (i.p.)	14.34±0.50	8.14±0.06	15 (136)
Cocaine (10 mg/kg, i.p)	13.27±0.60	8.17±0.08	6 (73)
Low-dose quinpirole (0.1 mg/kg, i.p.) + Cocaine (10 mg/kg, i.p.)	14.25±0.56	6.76±0.05***/°°°	7 (79)
High-dose quinpirole (0.5 mg/kg, i.p.)	11.29±0.82***/†††	7.34±0.08***/°°°	7 (63)
Low-dose quinpirole (0.1 mg/kg, i.p.)	10.80±0.73**/††	7.48±0.15***/°°°/†††	6 (24)

Nested ANOVA test ($F_{frequency}$ (4, 335) = 50.570, $p < 10^{-6}$; F_{power} (4, 335) = 4.634, $p < 10^{-2}$), followed by Tukey HSD post-hoc (*** $p < 10^{-3}$ for comparison to saline group; °°° $p < 10^{-3}$ for comparison to cocaine group; †† $p < 10^{-2}$, ††† $p < 10^{-3}$ for comparison to low-dose quinpirole + cocaine group)

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Discussion

Drug effects on the dominant frequency of theta oscillations and locomotor activity

Our results show that the injection of a high dose of D2/D3 agonist quinpirole, as well as co-injection of an autoreceptor-selective dose of quinpirole with a moderate dose of cocaine, produces a significant decrease in the dominant frequency of theta oscillations within the VTA network (Figures 2, 3). This electrophysiological effect is accompanied by a locomotor activation with prominent sniffing stereotypies in the case of high-dose quinpirole (Supplementary Video), and remarkable changes in the pattern of locomotion – locomotor stereotypy – in the case of low-dose quinpirole + cocaine (Figure 1 D). It could be argued that the increased number of completed circles after the co-injection of low-dose quinpirole and cocaine was a mere result of elevated locomotor activity rather than locomotor stereotypy. However, animals from the group treated by cocaine alone travelled similar distances and at similar speed, yet displayed no such circling behavior (Figure 1 B to D). Therefore, we conclude that the increase in circling is due to real changes in the quality of locomotion.

A moderate dose of cocaine alone, which may activate mainly D1 receptors, with little involvement of D2 receptors (Ferger et al., 1994), fails to induce a significant shift in the theta frequency. Behaviorally, the effect of cocaine appears as an increase of locomotor activity without clear signs of stereotypy.

It has to be emphasized that our results cannot be explained by a mere correlation between the LFP frequency and the speed of locomotion. Such correlation can be found for the data obtained from animals running on various types of run-ways and with various velocities (Slawinska and Kasicki, 1998). However, analyzing the short locomotor bouts of similar pattern, we still found a significant shift of theta frequency during the locomotor stereotypy provoked by the high quinpirole and quinpirole + cocaine injections (Table 2 and Figure 3).

Proposed mechanisms. Although speculative, the explanation of link between an altered frequency in the VTA and abnormal behavior can be found in a recently developed concept about the role of coherence in the communication between different neuronal groups. According to this concept, for effective communication between neuronal populations their activity should be phase-locked, i.e. have a consistent phase difference (see (Cannon et al., 2014; Fries, 2005; Varela et al., 2001) for review). ~~called "coherence filtering" (see (Cannon et al., 2014) for review). According to this concept, if area A projects to area B, with area B containing both "principal" (excitatory) neurons and inhibitory interneurons that are reciprocally interconnected, the input from A will be influencing B much more strongly if it is coherent with (has the same main frequency than) the major rhythm of area B.~~ Applying this concept to the current results would suggest that a distorted oscillation frequency in the VTA may profoundly modify its impact on postsynaptic target areas, such as the ventral striatum and hippocampus. Although very appealing, this hypothesis will have to be tested thoroughly in further experiments by simultaneous recordings from various areas. In addition, it is possible that similar alterations are produced in the substantia nigra and contribute to the change in behavior.

What could be the mechanism underlying the decrease of the dominant frequency of theta oscillations? At low doses quinpirole predominantly activates autoreceptors (Li et al., 1996; White and Wang, 1983), thereby suppressing the activity of those DA neurons that have a high somato-dendritic density of these receptors. This is known to be the case for a majority of midbrain DA neurons, except the mesoprefrontal ones (Chiodo et al., 1984; Ford and Williams, 2008; Lammel et al., 2008). Activation of these receptors is known to hyperpolarize the dendritic tree of DA neurons by opening G-protein-coupled K+ channels (Lacey et al., 1990). In addition, we have previously shown that activation of these and GABA-B receptors slows down or abolishes NMDA-induced membrane potential oscillations in DA neurons in midbrain slices (Seutin et al., 1994). The data of the present study are consistent with this finding, which could be the mechanism underlying the decrease of the

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dominant frequency of theta oscillations. Indeed, it is believed that fluctuations of dendritic membrane potentials are a significant component of delta/theta oscillations (Buzsaki et al., 2012). In addition, the observation that low-dose quinpirole decreases the main frequency of LFPs to a lesser extent (~15%), as compared to its effect on the firing rate of susceptible DA neurons in the same conditions (> 90%, Koulchitsky et al., 2012) may be related to the fact that mesoprefrontal DA neurons contribute significantly to the main rhythm of LFPs, thereby "diluting" the effect of the drug on the LFP frequency.

We cannot exclude the possibility that D2/D3 autoreceptors located on presynaptic DA terminals outside of the VTA contribute to the behavioral effect of low-dose quinpirole, and even to the change in LFPs. However, we do not favor this version because this dose of quinpirole was previously found to potently inhibit the firing of DA cells within this region (Koulchitsky et al., 2012) in the same experimental condition. Therefore, a predominant effect within the VTA is the most parsimonious explanation for our results.

Role of the theta frequency shift in the behavior. In general, our data support the current view about the role of the theta rhythm in the brain. According to it, theta oscillations control the timing of activity across neuronal populations in different brain areas playing a key role in the integration of sensory information with motor output (Bland and Oddie, 2001; Buzsaki and Draguhn, 2004; Hasselmo et al., 2002; Seidenbecher et al., 2003; Siapas et al., 2005). Even small shifts in the theta frequency might change the patterns of neural activity across the brain, leading to significant alterations in the emotional state and behavior (Dzirasa et al., 2009; Yamamoto, 1997, 1998). Thus, we believe that our electrophysiological results underlie the behavioral alteration that we observed, and may give some insight into mechanisms of locomotor regulation. We emphasize that these results do not allow us to define the direction of causality between the behavioral and electrophysiological events.

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Drug effects on the power of theta oscillations

We also observed a significant increase in the peak LFP power in the low-dose quinpirole + cocaine group, when comparing the averaged frequency power spectra corresponding to 20 min periods of recording (Table 1 and Figure 2). We suggest that the increased theta LFP power was due to the average increase in locomotion. Indeed, when calculating the frequency spectrum for the relatively long recording segments (20 min) we average frequency power values from the periods when the animal express locomotor activity (associated with the increase of theta oscillations) with those when the animal does not move, during which the theta rhythm is low or nearly absent. Because animals of the low-dose quinpirole + cocaine group spend more time moving than the other groups (Figure 1 A), they are expected to exhibit a higher theta power. This explanation is supported by the fact that this effect was absent when we compared LFP segments from the periods with similar locomotor patterns (Table 2 and Figure 3).

On the other hand, the short segment analysis revealed that low and high doses of quinpirole alone significantly decrease the power of theta oscillations (Table 2 and Figure 3). ~~We have currently no explanation for this effect which, although significant, was not very marked~~. This might be due to the fact that theta oscillations are usually associated more with the intention than with the movement itself (Whishaw and Vanderwolf, 1973; Wyble et al., 2004), and that the rise of theta power precedes the onset of voluntary locomotion (Vanderwolf, 1969). We noticed that animals in the saline, cocaine and quinpirole+ cocaine groups exhibit closely spaced successive locomotory episodes. With such a pattern, theta power from adjacent episodes might synergize. On the contrary, after the 0.1 mg/kg, and 0.5 mg/kg quinpirole injections the locomotory episodes were more sparse, and this potentiation may have been absent. However, this interpretation is still speculative at this stage.

Conclusion

We suggest that the alteration of the main frequency of the VTA rhythm induced by the action of quinpirole on D2 autoreceptors disturbs the ability of this region to interact with its input or output regions, hence the altered locomotor behavior. ~~This hypothesis is consistent with recent physiological and computational studies which have suggested that a critical parameter ensuring adequate communication between brain areas is coherence between their main rhythms (reviewed in (Cannon et al., 2014).~~ It will be interesting in future multisite recordings to test whether this change in frequency really leads to changes in synchrony with other brain structures.

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This study combines analysis of behavior and local field potentials in the rat ventral tegmental area after administration of dopaminergic drugs.

Co-administration of an autoreceptor-selective dose of quinpirole and cocaine leads to a very specific behavioral pattern consisting of locomotor stereotypies.

These stereotypies are accompanied by a change in the dominant frequency of the local field potentials.

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