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NMDA receptor antagonists ketamine and MK-801 induce wake-related aberrant γ oscillations in the rat neocortex

by

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ABSTRACT

Background: Single subanesthetic doses of ketamine, a non-competitive NMDA receptor (NMDAr) antagonist, induce cognitive impairment, schizophreniform psychosis, hallucinations, and exacerbate schizophrenia symptoms. The neuronal mechanisms underlying transient disruption in NMDAr function are unknown. Disorders of cognition-related coherences of γ frequency (30-80 Hz) oscillations between cortical areas are a major functional abnormality in schizophrenic patients. Does a single subanesthetic dose of ketamine or MK-801 alter properties of cortical γ oscillations?

Methods: Properties of spontaneously occurring γ oscillations in the electrocorticogram of the neocortex of freely moving rats (N=16) were measured before and after subcutaneous administration of a single dose of ketamine (≤ 10 mg/kg), MK-801 (≤ 0.16 mg/kg), d-amphetamine (≤ 1 mg/kg), apomorphine (≤ 1.6 mg/kg) or vehicle (NaCl, 0.9%).

Results: The present study gives the first evidence that ketamine and MK-801, both of which inducing NMDAr-dependent functional disconnections, dose-dependently increase the power (200-400%) of wake-related γ oscillations in the neocortex. Substances that modulate dopaminergic neurotransmission could also increase the γ power but to a lesser degree.

Conclusion: The present findings suggest that abnormal increased synchronization in ongoing γ oscillations in cortical-related networks might cause dysfunctions of conscious integration, as seen in patients with schizophrenia.

INTRODUCTION

Schizophrenia involves dysfunctions of conscious integration, which are thought to reflect functional disconnections in corticocortical systems (1,2). There is considerable evidence to suggest that N-methyl d-aspartate type glutamate receptors (NMDAr) are implicated in the pathophysiology of schizophrenia (3-6). Subanesthetic doses of non-competitive NMDAr antagonists induce cognition impairment, schizophreniform psychosis, hallucinations, and exacerbate symptoms in schizophrenic patients (7-10). The neuronal mechanisms underlying transient disruption in NMDAr function remain, however, to be determined. The cerebral cortex generates coherent synchronized γ frequency (30-80 Hz) oscillations during conscious brain operations (11). Disturbance of cognition-related coherences of γ oscillations between cortical areas is a major functional abnormality in schizophrenic patients (12). Therefore, the question arises as to whether a single subanesthetic dose of the NMDAr antagonists, ketamine or MK-801, alter properties of cortical γ oscillations. The aim of the present study was to assess the effect of a single dose of ketamine or MK-801 on spontaneously occurring γ oscillations in the neocortex of freely moving rats.

MATERIALS AND METHODS

Sixteen adult male Wistar rats (280-350 g) were used in this study. All animal care procedures were performed in accordance with local Ethical Committee and European Union guidelines (directive 86/609/EEC).

Drugs (dissolved in NaCl, 0.9%): Ketamine from Merial (Lyon, France), MK-801, d-amphetamine, apomorphine and physostigmine from Sigma-Aldrich (Saint-Quentin Fallavier, France), pentobarbital from Sanofi (Libourne, France).

Surgery: Rats were anesthetized (pentobarbital: 40 mg/kg, i.p. and ketamine : 50 mg/kg, i.m.) and positioned in a stereotaxic frame. Two stainless steel screws were implanted extradurally over the left and right frontoparietal cortices (from bregma: 1 mm anterior and 2 mm lateral). Two other screws were fixed in the frontal bone for ground connection and in the bone covering the cerebellum for reference. The screws were connected to a subminiature connector fixed to the skull with dental cement.

Recording, behavior and drug injection: Recording sessions began after one week recovery. The rats were connected to ultralow noise amplifiers (AI 402, x50; *Axon Instruments*) and had a 15 min habituation period before recording. The monopolar electrocorticogram (ECoG) of the left and right frontoparietal cortices was acquired 15 min prior to subcutaneous administration of a given substance. The bilateral ECoG was recorded for the next 70-90 minutes, which was repeated (2-3 days intervals) with different doses of a given drug (4-6 rats/drug). During the recording sessions animals were gently stimulated to ensure they did not fall asleep, and the behavior was rated every minute as immobile, active (exploration, eating, chewing, motion, grooming, etc.) ataxic or stereotyped. ECoG was processed with a bandpass of 0.1-1200 Hz and digitized at 2.5 kHz.

Data and statistical analysis: Fast Fourier Transformations (FFT) of ECoG recordings were computed using DataWave softwares (*SciWorks, v4, DataWave Technologie*). All the values of the power (1.6-s epochs) of γ oscillations were extracted before and after administration of a given substance. The 50 Hz values were discarded to avoid contamination from possible AC noise. The sum of the 30-49 Hz and 51-80 Hz values gave the total power. Data are presented as means \pm S.E.M. and compared with Student's t-test, two-way repeated-measures ANOVA, and with Tukey multiple comparison tests (confidence >0.95).

RESULTS

Features of wake-related cortical γ oscillations: The power and internal frequency of a given rhythm fluctuated at all times (Fig1D,E). Spontaneously-occurring γ frequency oscillations waxed and waned in amplitude over a period of about 100 ms (100.08 ± 4.06 ms; table 1). Each γ bout occurred at 2-4 Hz with maximal amplitude of 44.42 ± 1.34 μ V (Fig1A). Its averaged internal frequency at maximal power was 45.54 ± 0.77 Hz. The total power of γ oscillations (52.81 ± 0.59 μ V², N = 4398, 7 rats, >500 epochs from one ECoG electrode/rat) could significantly increase and decrease with action and immobility, respectively (Fig2).

Ketamine (2.5-10 mg/kg) or MK-801 (0.08-0.16 mg/kg) dose-dependently increases the power of γ oscillations in the rat (Figs1-3): This effect was accompanied by ataxic behavior (tottering, turning round on itself and falling down on its backside) (Fig2B-C), which was obviously more prominent at the highest doses. Both the maximal duration and the maximal amplitude of γ

bouts increased by two- to three-fold on average (Fig3; table 1). Their internal frequency at maximal power was significantly increased by about 10 Hz on average (table 1).

Repeated-measures ANOVA revealed significant effects of dose (and between doses), time and dose-time interaction (supplementary material1). Two features distinguished the kinetics of the ketamine and MK-801 effects on wake-related γ oscillations (Figs2&3): 1) The increase rate of the γ power was faster after ketamine injection than after MK-801 injection. Indeed, significant increase was observed as early as 1 and 15 minutes post-injection with all doses of ketamine and MK-801, respectively (Fig3, right panel). 2) Ketamine effects reached a maximum during the first 15 minutes then declined to partial or full recovery, depending on the dose, 70 minutes after injection, whereas MK-801-induced γ hyperactivity attained a maximal level during the first 20 minutes, which was still present 70 minutes after injection (Fig3, left panel).

Effects of substances that modulate dopaminergic neurotransmission: We also investigated the effects of substances that modulate dopaminergic neurotransmission on spontaneously occurring γ oscillations, since non-competitive NMDAr antagonists would also act as partial agonists of dopamine D2 receptors (13). Apomorphine and d-amphetamine are well known to induce locomotor activity and stereotyped behaviour and to mimic certain aspects of schizophrenia in animals. Subcutaneous injection of apomorphine (0.8-1.6 mg/kg, N = 4 rats) or d-amphetamine (0.5-1 mg/kg, N = 4 rats) induced locomotor hyperactivity and behavioral stereotypies accompanied by a slight increase (<200%) in the amount of cortical γ oscillations at the most (Fig4A-B). These effects lasted at least 60 minutes. After injection of apomorphine, a maximal increase of ~200% of the γ power, accompanied by an increase of behavioral hyperactivity, was observed at the end of the recording session in 2 out of 4 rats (Fig4A). Neither changes in the internal frequency at maximal power of γ oscillations nor apparent dose effect could be measured after injection.

DISCUSSION

This is the first study that demonstrates that ketamine or MK-801 dose-dependently increases the power (200-400%) of wake-related spontaneously occurring γ oscillations in the rat neocortex. The doses of ketamine administered (<5 mg/kg, sc) are almost equivalent to those (<1 mg/kg, iv) that induce cognitive deficits and a wide spectrum of behaviors relevant to schizophrenia in humans (7-

10). Furthermore, our doses are much smaller than the neurotoxic ones (ketamine>30 mg/kg, ip (14); MK-801=1 mg/kg, ip (15)) used in current animal models for schizophrenia (16).

The present experiments also show that ketamine- and MK-801-induced increased synchronization of γ oscillations is associated with abnormal (ataxic) motor activity. This behavior is quite similar to that seen during the earliest stage of anesthesia induction. Therefore, further experiments are needed to validate that non-competitive NMDAr antagonist-induced aberrant γ oscillations are not the consequence of premotor or motor activity. However, higher doses of ketamine (>10 mg/kg) provoke maximal increase of high-frequency (140-180 Hz) oscillations in the nucleus accumbens before the occurrence of maximal locomotor activity, suggesting that these ketamine-induced effects are not the consequence of motor action (17).

Is ketamine- or MK-801-induced increased γ synchrony specific for the blockage of NMDAr? Non-competitive NMDAr antagonists also bind at least at sigma (18) and dopaminergic (13) receptors. Our data show that apomorphine and d-amphetamine can increase the amount of γ oscillations, but to a much lesser extent than ketamine or MK-801, and without a dose-effect, suggesting that dopaminergic receptors have a minimal involvement in the generation of NMDAr antagonist-related γ hyperactivity. Further experiments are required to probe the effects of sigma receptors. Furthermore, cholinergic receptors would not be involved in the generation of the γ hyperactivity (supplementary material2). At the very low concentrations used, ketamine and MK-801 are expected to act first on inhibitory local circuit neurons since they are tenfold more sensitive to NMDAr antagonists than pyramidal neurons (19).

Gamma frequency oscillations critically depend upon parvalbumin expressing GABAergic interneurons, which are involved in recurrent perisomatic inhibition of pyramidal cells (20) and which are particularly affected in schizophrenic patients (21). Furthermore, the number of GABAergic interneurons that express NMDAr is significantly decreased in schizophrenic patients (6) and in a pharmacological model for psychosis (5). These and other (22-25) findings strongly support the hypothesis that NMDAr-dependent reduced recurrent inhibition is involved in the pathophysiology of schizophrenia. It is known that MK-801 and phencyclidine modulate the firing rate in prefrontal cortex neurons (26-27). Further studies are necessary to understand the link between NMDAr hypofunction and aberrant γ oscillations in the animal model for psychosis that is described here.

Increased and decreased synchronizations in γ oscillations have been recorded in schizophrenic patients, depending on the cortical region and the symptoms (28-31). Interestingly, increased γ

synchronization has been recorded in patients with somatic (32) and visual (31) hallucinations. Therefore it is tempting to speculate that increased synchronization in spontaneously occurring γ oscillations in cortical-related networks might be a cause of the dysfunctions of conscious integration during psychotic symptoms, as seen in patients with schizophrenia.

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FINANCIAL DISCLOSURES

The author has no conflicts of interests to declare.

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| | control (NaCl, 0.9 %) | ketamine (10 mg/kg) ~15 minutes | MK-801 (0.16 mg/kg) ~30 minutes |
|---|--------------------------|------------------------------------|------------------------------------|
| duration (ms) | 100.08 ± 4.06 (N=120) | 238.17 ± 3.07 (N=120) P<0.001 | 145.25 ± 3.92 (N=120) P<0.001 |
| amplitude (μV) | 44.42 ± 1.34 (N=120) | 109.00 ± 3.21 (N=120) P<0.001 | 105.56 ± 3.87 (N=120) P<0.001 |
| internal frequency at maximal power (Hz) | 45.54 ± 0.77 (N=80) | 54.35 ± 0.51 (N=80) P<0.001 | 58.25 ± 0.85 (N=80) P<0.001 |

Table 1: Significant changes of the properties (means ± S.E.M.; Student's t-test) of wake-related, spontaneously occurring γ oscillations after subcutaneous administration of ketamine or MK-801 compared to the vehicle (NaCl, 0.9 %).

FIGURE LEGENDS

Table 1: Significant changes of the properties (means \pm S.E.M.; Student's t-test) of wake-related, spontaneously occurring γ oscillations after subcutaneous administration of ketamine or MK-801 compared to the vehicle (NaCl, 0.9 %).

Figure 1: Ketamine or MK-801 increases the power of spontaneously occurring γ oscillations. **A-C:** 1-s episode of a wake-related bilateral ECoG containing γ oscillations under control conditions (NaCl, 0.9 %; A), 15 min after ketamine injection (B), and 30 min after MK-801 injection (C). **D-E:** Spectral analysis of 20 successive 1.6-s episodes (total: 32 s; resolution: 5 Hz) is shown either 5 min before, 15 and 65 min after ketamine injection (D), or 5 min before, 30 and 65 min after MK-801 injection (E). The histograms in (D) and (E) show the corresponding means (\pm S.E.M., N = 20) of the total power of γ oscillations before and after administration of ketamine or MK-801, which are compared using Student's t test (*, $p < 0.001$). Note partial recovery of the amount of γ oscillations 65 min after injection. Abbreviation: sc, subcutaneous.

Figure 2: Ketamine- or MK-801-induced abnormal γ hyperactivity is associated with abnormal behaviour. Each chart represents a recording session during which the rat received an injection at 15 min (arrow). The power (in black) of γ oscillations fluctuates during time with behaviour (3-colours-gray scale; see methods and result sections for details). **A:** Under control conditions (NaCl, 0.9 %), γ power could increase when the animal is active (exploration, chewing, motion, grooming, etc.). Note that the power in average trends to slightly decrease during the recording session. **B:** A few days later, the rat received an injection of ketamine. Note the transient dramatic increase in the power of γ oscillations, which is accompanied by an abnormal (ataxic) motor behaviour. **C:** Another rat received an injection of MK-801. Note that the increase in power of γ oscillations is still present 75 minutes after injection, which is accompanied by ataxic behaviour. Abbreviation: sc, subcutaneous.

Figure 3: Ketamine or MK-801 dose-dependently increases the amount of cortical γ oscillations. Ketamine (2.5, 5 or 10 mg/kg), MK-801 (0.08 or 0.16 mg/kg) or the vehicle (NaCl 0.9 %, 1 ml/kg; = control) was administered subcutaneously (sc, at time 0) after a 15-min habituation period and a 15-min period of acquisition (5 rats per group). All values are normalized. Each control value (100 % change) corresponds to the mean of 5 rats (> 30 individual FFT values/rat sampled during the previous 5 minutes). At a given time (± 15 sec), each value in the chart corresponds to the

mean of 5 rats (> 10 individual FFT values/rat). On the right, are expanded the first 15 minutes. The gray areas indicate the time from which (ketamine: 1 min; MK-801: 15 min) the means are significantly different (two-way repeated-measures ANOVA and Tukey multiple comparison tests, confidence > 0.95; see supplementary material1).

Figure 4 : Effects on the power of γ oscillations of the activation of dopaminergic neurotransmission. Each chart represents a recording session during which the rat received an injection at 15 min (arrow). The power (in black) of γ oscillations fluctuates during time with behaviour (3-colours-gray scale; see methods and result sections for details). In the frontoparietal cortex of freely moving rats, at doses that induce locomotor activity and behavioral stereotypies, apomorphine (A) or d-amphetamine (B) trended to slightly increase the amount of γ oscillations. The rat treated with apomorphine (A) was, in addition, very hyperactive during the last 10 minutes of the recording session. Amphetamine maintains the level of γ power at or just above the control level (B, compare with control, Fig2A).

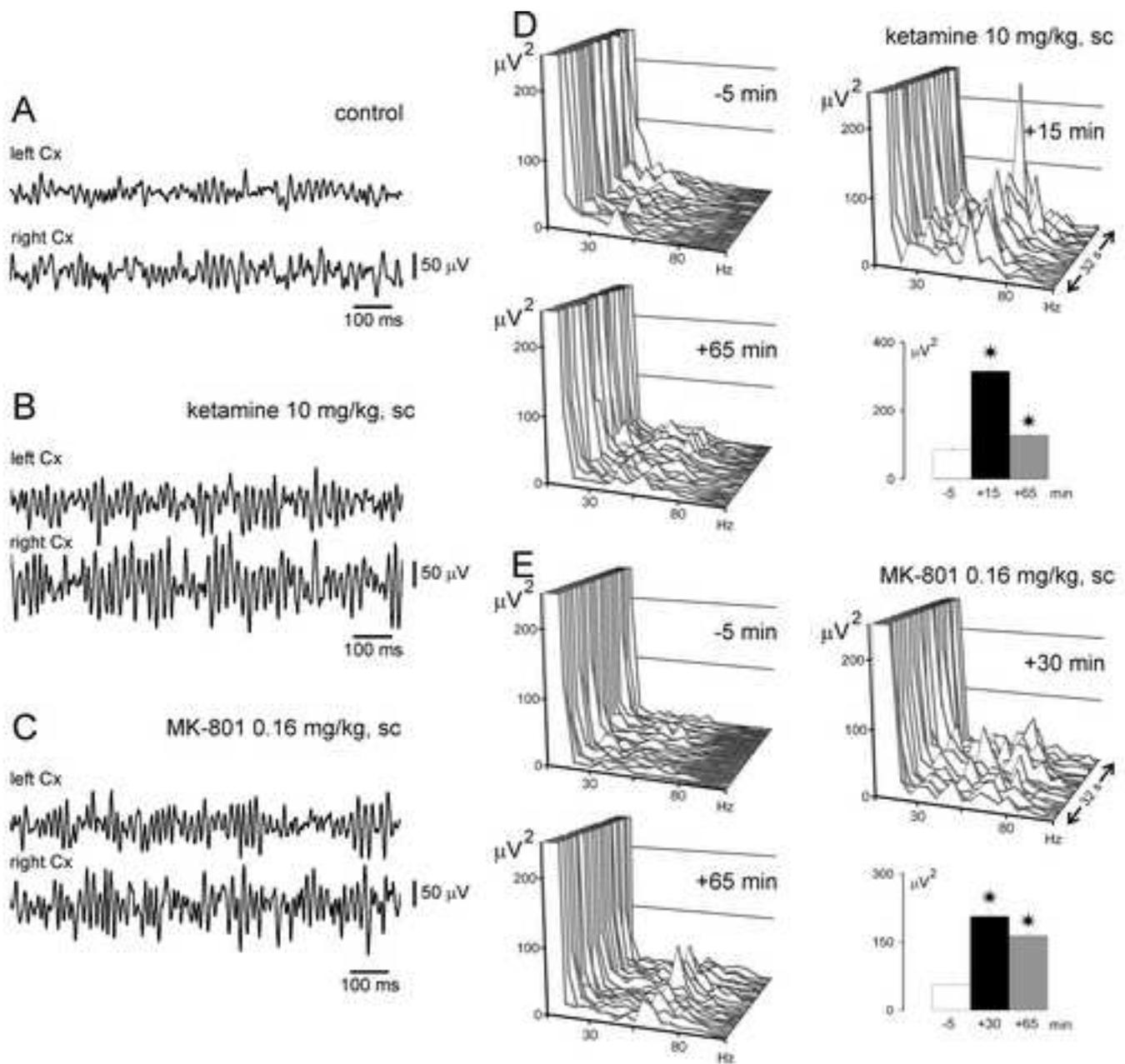


Figure 1

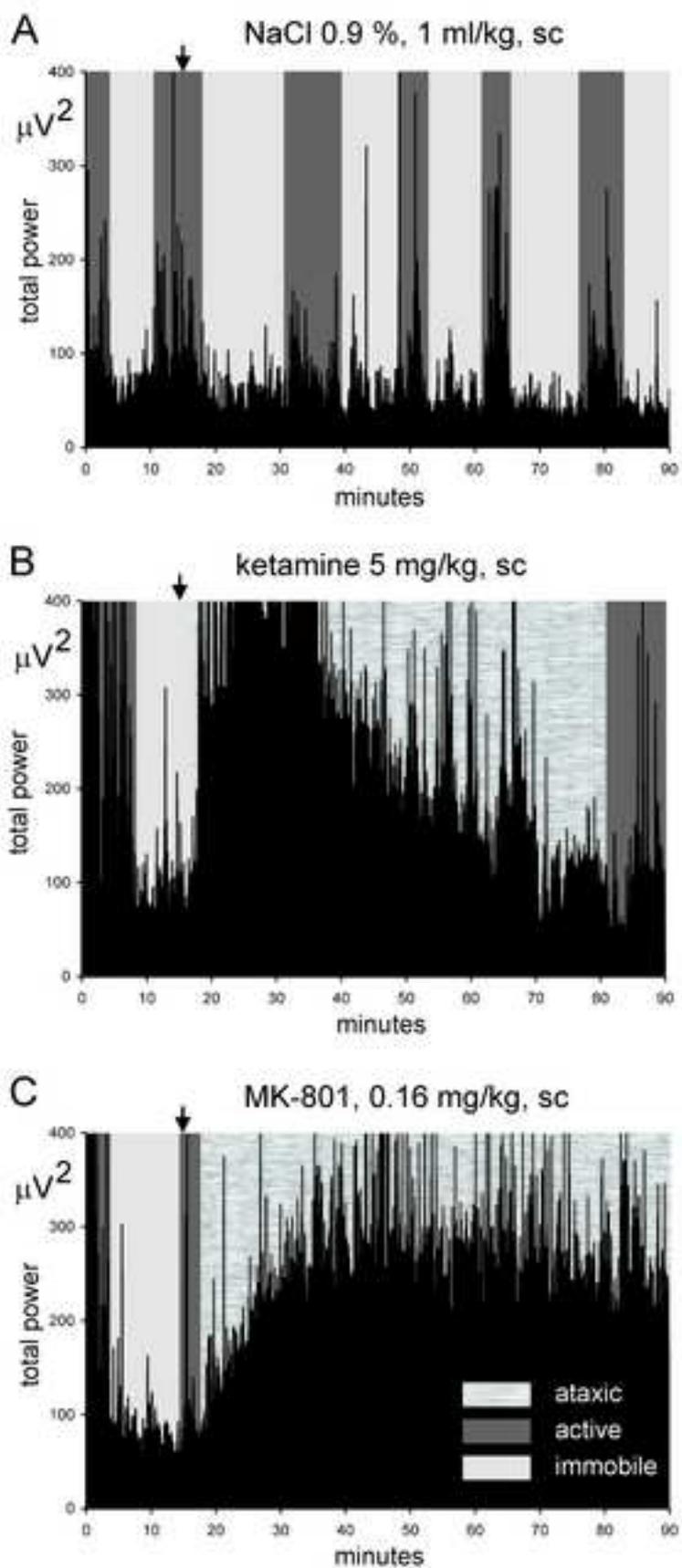


Figure 2

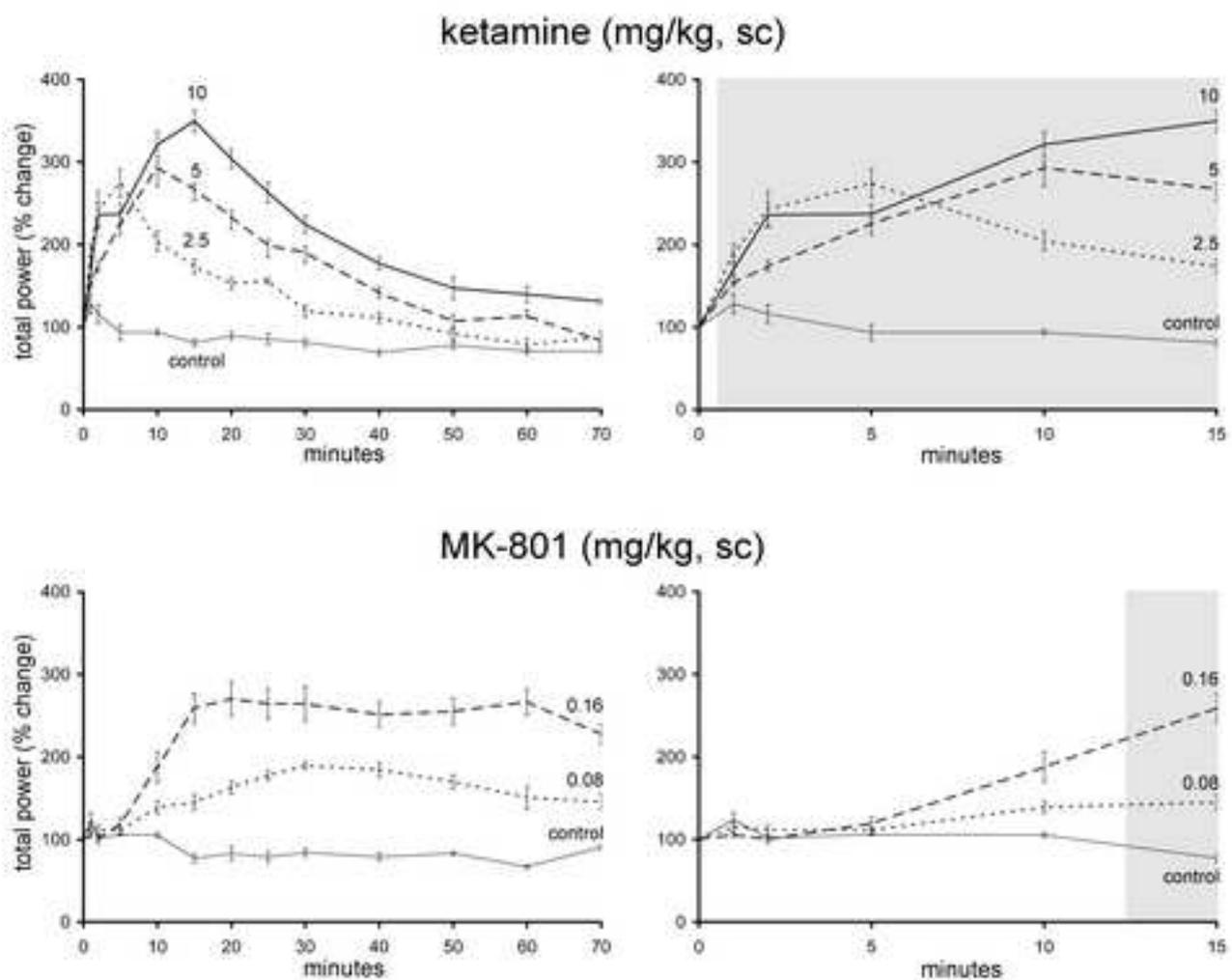


Figure 3

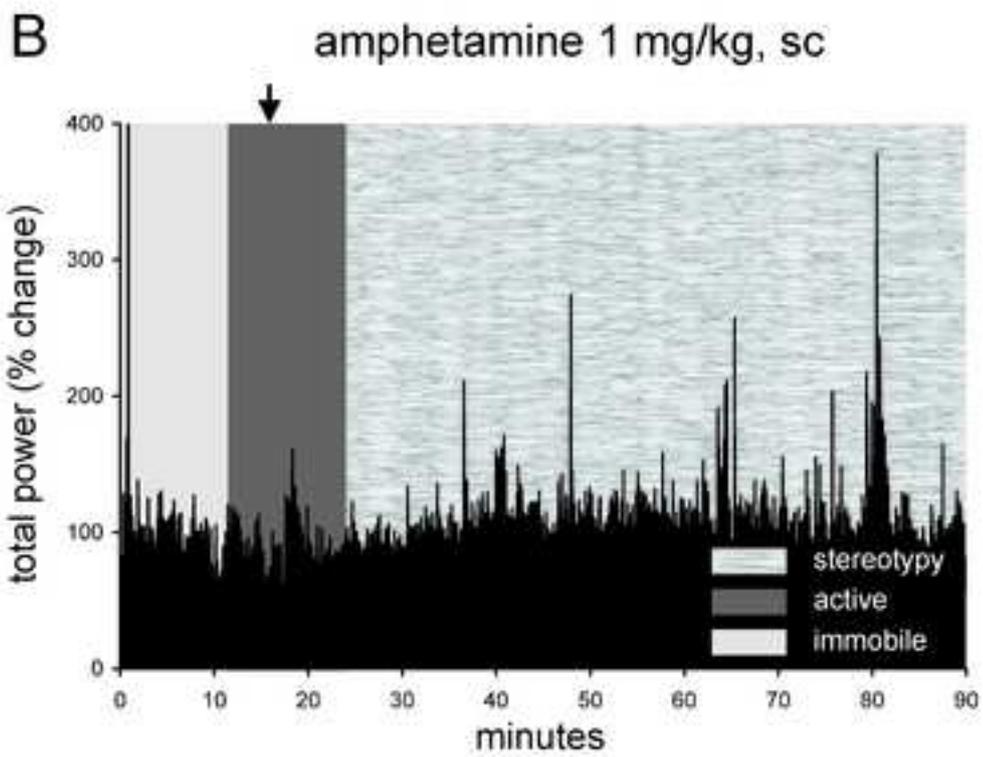
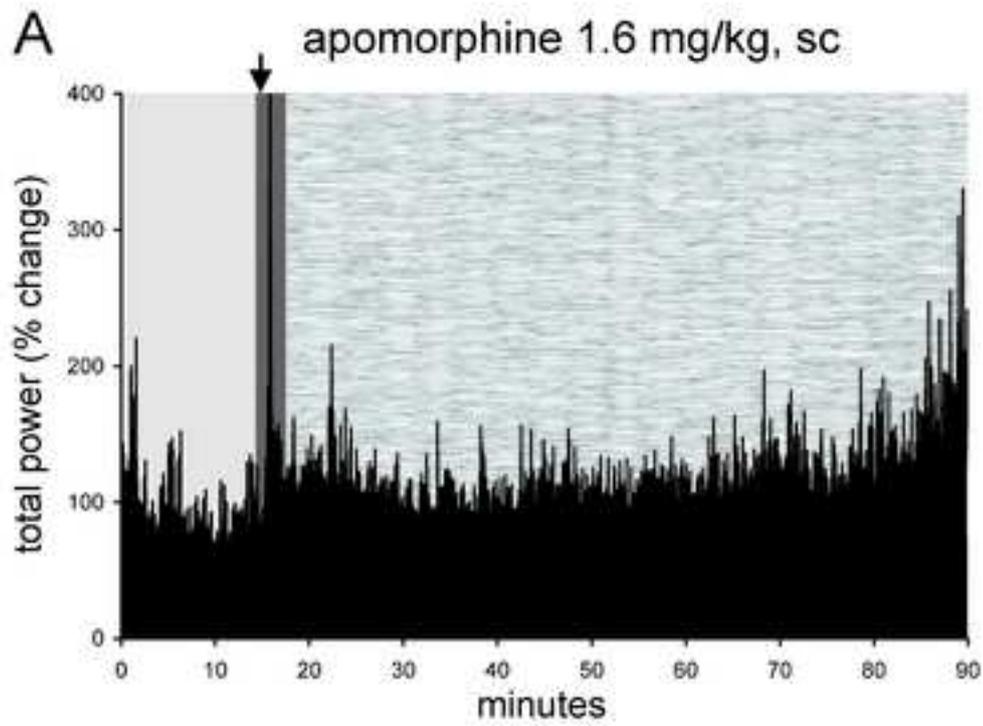


Figure 4

SUPPLEMENTARY MATERIAL

| | KETAMINE | MK-801 |
|-----------|---------------------------------|---------------------------------|
| dose | F(3,16) = 28.699 P < 0.001 | F(2,12) = 19.1057 P < 0.001 |
| time | F(11,176) = 32.926 P < 0.001 | F(11,132) = 13.810 P < 0.001 |
| dose*time | F(33,176) = 6.094 P < 0.001 | F(22,132) = 11.435 P < 0.001 |

Table 2 (see Fig3) : Two-way repeated-measures ANOVA showing significant effects of dose, time and dose-time interaction of ketamine and MK-801 in the power of cortical γ oscillations.

| time (min) | KETAMINE | MK-801 |
|------------|-----------|-----------|
| 1 | P < 0.001 | P = 0.999 |
| 2 | P < 0.001 | P = 1.000 |
| 5 | P < 0.001 | P = 1.000 |
| 10 | P < 0.001 | P = 0.117 |
| 15 | P < 0.001 | P = 0.002 |
| 20 | P < 0.001 | P < 0.001 |
| 25 | P < 0.001 | P < 0.001 |
| 30 | P < 0.001 | P < 0.001 |
| 40 | P = 0.795 | P < 0.001 |
| 50 | P = 1.000 | P < 0.001 |
| 60 | P = 1.000 | P = 0.002 |
| 70 | P = 1.000 | P = 0.011 |

Table 3 (see Fig3) : Tukey multiple comparison tests showing the period (comparison with time 0 min) during which (ketamine: 1-30 min; MK-801: 15-70 min) means of cortical γ power significantly differ (confidence > 0.95; gray).

| dose (i) mg/kg, sc | dose (j) mg/kg, sc | KETAMINE | MK-801 |
|--------------------|--------------------|-----------|-----------|
| 0 | 2.5 | P < 0.001 | na |
| 0 | 5 | P < 0.001 | na |
| 0 | 10 | P < 0.001 | na |
| 2.5 | 5 | P = 0.008 | na |
| 2.5 | 10 | P < 0.001 | na |
| 5 | 10 | P < 0.001 | na |
| 0 | 0.08 | na | P < 0.001 |
| 0 | 0.16 | na | P < 0.001 |
| 0.08 | 0.16 | na | P < 0.001 |

Table 4 (see Fig3) : Tukey multiple comparison tests showing significant differences between doses of ketamine or MK-801 in the cortical γ power (confidence > 0.95; gray). Abbreviation: sc, subcutaneous.

SUPPLEMENTARY MATERIAL

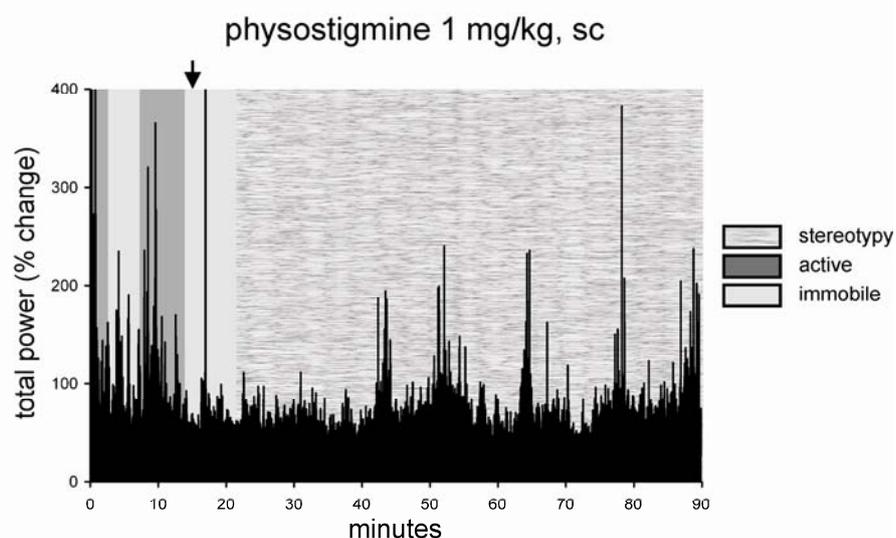


Figure 5 : Effects on the power of γ oscillations of the activation of cholinergic neurotransmission. The chart represents a recording session during which the rat received a subcutaneous (sc) injection of physostigmine at 15 min (arrow). The power (in black) of γ oscillations fluctuates during time with behaviour (3-colours-gray scale; see methods and result sections for details). In the frontoparietal cortex of freely moving rats, at doses that induce locomotor activity and behavioral stereotypes, physostigmine did not significantly increase the amount of γ oscillations during the recording session.

Gamma oscillations can be provoked *in vitro* by cholinergic agonist carbachol (Fishan et al., 1998). Therefore the effect of injections of physostigmine, a reversible cholinesterase inhibitor that indirectly stimulates muscarinic and nicotinic receptors (Pope et al., 2005), on spontaneously occurring γ oscillations was examined. Even with doses (1 mg/kg; N = 4 rats) that induced behavioral stereotypes, the spontaneous changes in the γ power over time (Fig 5) were similar to those observed in control rats (see Fig 2A). This result suggests that cholinergic receptors were not involved in the generation of NMDAR antagonist-related γ hyperactivity.

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