Behavioral/Cognitive

Too Little and Too Much: Hypoactivation and Disinhibition of Medial Prefrontal Cortex Cause Attentional Deficits

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Attentional deficits are core symptoms of schizophrenia, contributing strongly to disability. Prefrontal dysfunction has emerged as a candidate mechanism, with clinical evidence for prefrontal hypoactivation and disinhibition (reduced GABAergic inhibition), possibly reflecting different patient subpopulations. Here, we tested in rats whether imbalanced prefrontal neural activity impairs attention. To induce prefrontal hypoactivation or disinhibition, we microinfused the GABA-A receptor agonist muscimol (C4H6N2O2; 62.5, 125, 250 ng/side) or antagonist picrotoxin (C30H34O13; 75, 150, 300 ng/side), respectively, into the medial prefrontal cortex. Using the five-choice serial reaction time (5CSRT) test, we showed that both muscimol and picrotoxin impaired attention (reduced accuracy, increased omissions). Muscimol also impaired response control (increased premature responses). In addition, muscimol dose dependently reduced open-field locomotor activity, whereas 300 ng of picrotoxin caused locomotor hyperactivity; sensorimotor gating (startle prepulse inhibition) was unaffected. Therefore, infusion effects on the 5CSRT test can be dissociated from sensorimotor effects. Combining microinfusions with in vivo electrophysiology, we showed that muscimol inhibited prefrontal firing, whereas picrotoxin increased firing, mainly within bursts. Muscimol reduced and picrotoxin enhanced bursting and both drugs changed the temporal pattern of bursting. Picrotoxin also markedly enhanced prefrontal LFP power. Therefore, prefrontal hypoactivation and disinhibition both cause attentional deficits. Considering the electrophysiological findings, this suggests that attention requires appropriately tuned prefrontal activity. Apart from attentional deficits, prefrontal disinhibition caused additional neurobehavioral changes that may be relevant to schizophrenia pathophysiology, including enhanced prefrontal bursting and locomotor hyperactivity, which have been linked to psychosis-related dopamine hyperfunction.

Key words: attention; cognitive deficits; disinhibition; hypoactivation; in vivo electrophysiology; prefrontal cortex

Introduction

Cognitive deficits, including attentional deficits, pose a major treatment challenge in many neuropsychiatric diseases, including schizophrenia (Millan et al., 2012). In schizophrenia, such deficits have emerged as a core feature of the illness and a major determinant of disability (Green and Nuechterlein, 1999) and they are resistant to current treatments (Keefe et al., 2007). To develop efficient treatments, it is important to identify neural mechanisms underlying cognitive deficits.

Dysfunction of the prefrontal cortex is an important candidate mechanism for attentional deficits, given this region’s key role in attention (Dalley et al., 2004; Chudasama and Robbins, 2006). In schizophrenia, one important line of evidence points to prefrontal hypoactivation (i.e., reduced activation). Functional imaging studies have shown prefrontal hypoactivation, especially a reduced task-related activation of the dorsolateral prefrontal cortex in parallel with impaired task performance, even though concerns have been raised that hypoactivation may be a consequence, rather than a cause, of poor performance (Weinberger and Berman, 1996; Minzenberg et al., 2009; Ortiz-Gil et al., 2011). Another convincing line of evidence points to prefrontal disinhibition (i.e., reduced GABAergic inhibition). Key evidence comes from postmortem neuropathological findings of altered prefrontal GABAergic markers (Beasley et al., 2002; Lewis and Moghaddam, 2006; Fung et al., 2010), with recent evidence suggesting that subsets of patients differ in the severity of GABAergic dysfunction (Volk et al., 2012). Therefore, there is evidence for both prefrontal hypoactivation and prefrontal disinhibition in schizophrenia, potentially reflecting distinct patient subsets. It is also possible that task-related hypoactivation (i.e., a reduced activation difference between task and off-task condition) may reflect disinhibited off-task activity to irrelevant stimuli. Balanced prefrontal neural activity (i.e., both appropriate activation in response to relevant stimuli and inhibition of responses to irrele-
vant stimuli) may be important for prefrontal-dependent cognitive function (Goldman-Rakic, 1995; Rao et al., 2000). Therefore, prefrontal hypoactivation and disinhibition may both cause deficits in prefrontal-dependent cognition. However, the correlative clinical evidence does not establish such a causal relationship.

Here, we tested in rats the hypothesis that balanced prefrontal activity is important for attention. We used the GABA-A agonist muscimol (C4H6N2O2) to temporarily reduce prefrontal neural activation, i.e. to induce prefrontal hypoactivation, and the GABA-A antagonist picrotoxin (C30H32O5S) to temporarily reduce GABAergic inhibition, i.e. to induce disinhibition. The drugs were microinfused into the medial prefrontal cortex, which shares functional-anatomical properties with the human doro-

lateral prefrontal cortex (Uylings et al., 2003). To measure attention, we used the five-choice-serial-reaction-time (5CSRT) task. This task, which also provides parallel measures of response control, is prefrontal dependent and resembles human continuous performance tasks (Robbins, 2002; Chudasama and Robbins, 2006; Lustig et al., 2012), which have been widely used to measure attentional deficits in schizophrenia (Cornblatt and Keilp, 1994) and on which patients show prefrontal hypoactivation (Buchsbaum et al., 1990; Volz et al., 1999). For comparison, we also included locomotor and startle prepulse inhibition (PPI) testing, with locomotor hyperactivity and PPI disruption being widely used psychosis-related indices (Bast and Feldon, 2003; Arguello and Gogos, 2006; Swerdlow et al., 2008). Importantly, to link cognitive/behavioral effects to neural changes, we characterized how muscimol and picrotoxin altered prefrontal neural activity using multiunit and local field potential (LFP) recordings.

Materials and Methods

Subjects

Adult male Lister hooded rats (Charles River Laboratories) were used for all experiments: 24 rats for the 5CSRT experiments (~6 months old at surgery), 60 rats for the locomotor and PPI experiments (~2–3 months old at surgery), and 26 rats for the electrophysiology (~2–3 months old at the time of the acute experiment). The age at which rats were tested in the different experiments was an important consideration when planning our studies. Although the rat prefrontal cortex matures postnatally, available evidence suggests that this maturation, including the matura-

tion of the GABAergic system and of its modulation, is complete in rats that are 2–3 month of age (i.e., postnatal day 60–90; Benes et al., 2000; Tseng and O’Donnell, 2007; Le Magueresse and Monyer, 2013); of partic-

ular relevance to the present study, experiments examining matura-

tion of prefrontal GABAergic transmission showed that this maturation is complete in rats that are 65–85 d old (and possibly earlier; Caballero et al., 2013; Thomases et al., 2013). Therefore, and considering that keeping rats for an additional 3–4 months in captivity has both cost and animal welfare implications, we concluded that it was appropriate to conduct the sensorimotor and electrophysiological experiments in young adult rats (2–3 months, i.e., 60–90 d) and that the findings can be used to interpret the effects in the 5CSRT experiments where rats were tested at an age of ~6 months. Rats were housed in cages of 4 under temperature-controlled conditions and alternating 12 h light and 12 h dark cycle (lights on 7:00–19:00). Rats had ad libitum access to water and food (Teklad Global 18% Protein Rodent Diet 2018; Harlan Laboratories), except for the 24 rats used for the 5CSRT experiments, which had restricted access to food during behavioral testing. Those rats received a restricted amount of food (at least 18 g per day, but more if rats fell below target weights) to maintain them at 80–85% of their free-feeding weight estimated according to a preestablished weight growth curve. All rats were habituated to handling by the experimenters before the start of any experimental procedures. Experimental procedures were always con-

ducted during the light phase if at all possible. All procedures were con-

ducted in accordance with the requirements of the UK Animals

(Scientific Procedures) Act of 1986. All efforts were made to minimize suffering and to reduce the number of animals used.

Implantation of guide cannulae into the medial prefrontal cortex for behavioral studies

Rats were anesthetized using isoflurane delivered in oxygen (induction: 4–5%; maintenance: 1–3%) and were secured in a stereotaxic frame. To minimize the risk of pain, EMLA cream (5%, AstraZeneca) was used on the ear bars, lidocaine (4% w/v; South Devon Healthcare) was applied to the incision site on the scalp, and the rats received perioperative analgesia (Rimadyl large animal solution, 2:10 dilution, 0.1 ml/200 g, s.c.). The skull was exposed and bregma and lambda were aligned horizontally. Bilateral infusion guide cannulae (the “mouse” model C233GS-5.1-2; Plastic Ones) consisting of a 5 mm plastic pedestal that held 2 26 gauge metal tubes, 1.2 mm apart and projecting 4.5 mm from the pedestal, were implanted through small holes drilled in the skull. The tips of the guide cannulae were aimed 0.5 mm above the injection site in the prelimbic prefrontal cortex at the following coordinates: 3 mm anterior and 0.6 mm lateral from bregma and 3.5 mm ventral from the skull surface. These coordinates were adapted from a previous study (Marquis et al., 2007) on the basis of pilot surgeries. Cannulae were secured to the skull with dental acrylic and stainless steel screws. Double stylets (33 gauge; Plastic Ones) were inserted into the guides (with no protrusion) and the guides were closed with a dust cap. After surgery, the rats were allowed at least 5 d of recovery before any testing commenced. During the recovery period, rats were checked daily and habituated to the manual restraint necessary for the drug microinfusions.

Microinfusion procedure and drugs for behavioral studies

Rats were gently restrained and 33 gauge injectors (Plastic Ones) were inserted into the guides. The injector tips extended 0.5 mm below the guides into the medial prefrontal cortex and the injector ends were con-

nected through polyethylene tubing to 5 μl syringes mounted on a mi-

croinfusion pump. A volume of 0.5 μl/side of 0.9% sterile saline (control) or of a solution of muscimol or picrotoxin in saline was then infused bilaterally over 1 min (for concentrations, see specific experi-

ments). The movement of an air bubble, which was included in the tubing, was monitored to verify that liquid was successfully infused into the brain. The injector remained in place for 1 additional minute to allow for tissue absorption of the infusion bolus. The injectors were then re-

moved and the stylets replaced. Testing started 10 min after the infusion, except for locomotor testing, which commenced as soon as possible after the infusion (with postinfusion locomotor testing lasting 60–90 min and yielding a time course measure; this allowed us to determine the onset of the drug effect).

Picrotoxin and muscimol (Sigma-Aldrich) were dissolved in saline at concentrations of 150 or 300 ng/0.5 μl and 1 μg/μl, respectively. These solutions were aliquoted and kept frozen until use (not longer than 1 year). On the day of infusion, aliquots were thawed and, if necessary, diluted to the required concentration with saline.

Visual inspection for behavioral seizure signs

Given the potential of GABA-A antagonists to induce seizures (Neckel-
mann et al., 1998; Steriade and Contreras, 1998; Castro-Alamancos, 2000; Brgin et al., 2009), all rats were carefully monitored for behavioral indicators of seizure development after infusions and between infusion days. None of the infusions, including the picrotoxin infusions, induced motor convulsions or more subtle effects that may point to seizure de-

velopment, such as facial twitches, tremor, movement arrest, or wet-dog shakes (Brgin et al., 2009).

5CSRT experiments

The 5CSRT test requires rats to sustain and divide attention across a row of 5 apertures to detect brief (0.5 s) light flashes occurring randomly in one of the apertures and to respond to these flashes by nose-poking into the correct hole to receive food reward. Our procedures were adapted from previous studies (Pezze et al., 2007; Bari et al., 2008; Pezze et al., 2009).
Test sessions and performance measures

A test session started with the delivery of a free food pellet into the magazine, after which the rat could trigger a trial by nose-poking into the food magazine. The trial would start after a 5 s delay (intertrial interval; ITI), with a light going on in one of the apertures for a stimulus duration (SD) of 0.5 s. If the rat nose-poked into that aperture within a limited hold (LH) period of 5 s (correct response), a reward pellet was released into the food magazine. Responses in one of the unlit four holes (incorrect response), failure to respond within the LH period (omission), and responses during the ITI (premature response) were punished by a 5 s time-out period, during which the house light was turned off. Repeated responses in either the correct or an incorrect aperture were recorded as perseverative responses. The next trial was triggered when the rat entered the food magazine either to collect the reward or after the 5 s time-out; once triggered, a new trial would start after a 5 s ITI. The order in which apertures were lit across trials was random. Test sessions consisted of 100 trials (presentations of light stimulus) or lasted 30 min, whichever was shorter. Each rat had only one test session per day.

The following performance measures were analyzed (compare Robins, 2002; Amitai and Markou, 2010):

1. Measures of attentional performance: percentage accuracy ([correct responses/(correct responses + incorrect responses)] * 100%), reflecting errors of commission due to faulty stimulus detection independent from errors of omission; percentage omissions ([omissions/(correct responses + incorrect responses + omissions)] * 100%), which may reflect failure to detect the stimulus, but could also reflect motor and/or motivational deficits, depending on additional measures (see #3 below).

2. Measures of response control: percentage premature responses ([premature responses/(correct responses + incorrect responses + omissions + premature responses)] * 100%) and percentage perseverative responses ([perseverative responses/(correct responses + incorrect responses + omissions + premature responses)] * 100%), reflecting failure to withhold prepotent, but inappropriate, responses.

3. Additional measures: number of trials, correct response latency (mean duration between stimulus onset and nose poke in correct hole), and collect latency (mean duration between nose poke in correct hole and collection of reward in food magazine); nonspecific motor and/or motivational changes would be reflected by global changes in these measures.

Habitation, shaping, and training to high and stable performance levels before surgery

Before being subjected to the test sessions described in the previous section, rats were first habituated to the boxes and shaped to perform nose-poke responses. Testing started at day 2 and the other half on day 4. Mock infusions were performed in the same way as the microinfusions (see above, Microinfusion procedure and drugs for behavioral studies) except that tubing and injection cannulae were empty. The purpose was to habituate rats to the infusion procedure and to avoid interference with task performance.

The effects of pretreatment with muscimol or picrotoxin infusions: experimental design

After at least 5 d of recovery from surgery, rats were first retrained to perform at criterion level for at least 5 consecutive days. They then underwent 4 d of testing, with half of the rats receiving mock infusions on day 2 and the other half on day 4. Mock infusions were performed in the same way as the microinfusions (see above, Microinfusion procedure and drugs for behavioral studies) except that tubing and injection cannulae were empty. The purpose was to habituate rats to the infusion procedure and to avoid interference with task performance.

The effects of pretreatment with muscimol or picrotoxin infusions were then tested in separate within-subjects studies, with testing order of the different drug doses counterbalanced using a Latin-square design and each infusion day preceded by a testing day without infusions (to assess normal performance off-drug and avoid carry-over effects). Studies involving muscimol and picrotoxin infusions were both run in 2 replications (n = 5–7 per replication, n = 12 across the 2 replications). Each replication included testing of two different dose ranges (saline plus two doses of 40 and 80 μg for muscimol and 40 and 120 μg for picrotoxin).
either muscimol or picrotoxin; i.e., each rat received a total of six infusions). Test sessions started 10 min after the infusion. Testing of the 2 dose ranges was separated by 5 d of testing without infusion (to reestablish a stable baseline) and the testing order for the 2 dose ranges was swapped between the 2 replications. One common dose was included in both dose ranges as an internal control for the reproducibility of the drug effects.

Muscimol. The first replication included seven rats that were first tested with a dose range including saline, 125 and 250 ng/side muscimol (based on Marquís et al., 2007), and then with a lower dose range including saline and 62.5 and 125 ng/side (because we found very strong effects with the higher doses); the second replication included five rats that were first tested with the lower dose range and then with the higher dose range.

Picrotoxin. The first replication included seven rats that were first tested with saline and 75 and 150 ng/side picrotoxin (based on our previous studies involving hippocampal picrotoxin infusions; Bast et al., 2001) and then with a higher dose range including saline and 150 and 300 ng/side (because we did not find effects on the 5CSRTT with the lower doses); the second replication included five rats that were first tested with the higher dose range and then with the lower dose range.

Sensorimotor testing: startle PPI and open-field locomotor activity

For comparison with the effects of prefrontal muscimol and picrotoxin on attention and response control and to address whether these effects may be related to changes in basic sensorimotor processes, we examined the effects of these prefrontal manipulations on PPI of the acoustic startle response and on locomotor activity. PPI and locomotor testing are widely used in preclinical schizophrenia research, because disrupted PPI and increased locomotor activity are often used as simple psychosis-related indices in rodents (Bast and Feldon, 2003; Arguello and Gogos, 2006). PPI refers to the reduction of the startle response to an intense acoustic pulse by a weaker, nonstartling prepulse that shortly precedes the startle pulse. It may reflect sensorimotor gating processes and tends to be disrupted in schizophrenia and to be ameliorated by antipsychotic medication, even though PPI disruption is not specific to schizophrenia and the functional and clinical relevance of PPI reduction remains to be clarified (Swerdlow et al., 2008). Locomotor hyperactivity may often reflect dopamine hyperfunction, similar to psychosis (Bast and Feldon, 2003).

Startle and PPI

Measurements were conducted using four startle response systems (San Diego Instrument) similar to previous studies (Jones et al., 2011). Each system was placed inside a well lit (15 W) and ventilated sound attenuated chamber (39 × 38 × 58 cm³) and consisted of a clear Perspex cylinder (8.8 cm diameter, 19.5 cm long) on a solid Perspex base linked to an on an accelerometer. Background noise and acoustic stimuli were produced by a noise generator controlled by the SR-Lab system (San Diego Instrument) and presented by a speaker located centrally above the cylinder. Individual whole-body startle responses were recorded by the accelerometer connected to Reflex Testing software (San Diego Instruments). The amplitude of the whole-body startle response to an acoustic pulse was defined as the average of 100 1 ms accelerometer readings collected from pulse onset.

A test session started with the rat being put into the cylinder for a 5 min acclimatization period with a 62 dB(A) background noise level that continued through the session. After the acclimatization period, there were three test blocks. In the first block, 10 startle pulses (40 ms, 120-dB(A) broad-band bursts) were presented alone, so that the startle response could habituate to a relatively stable level of startle reactivity for the remainder of the test session. The second block consisted of 50 trials to measure PPI. There were five different trial types, each presented 10 times, in pseudorandom order and with a variable intertrial interval of 10 to 20 s duration (average 15 s), so as to be unpredictable: pulse-alone trials and four types of prepulse-plus-pulse trials in which a weak 20 ms prepulse (72, 76, 80, or 84 dB(A)) preceded the startle pulse by 100 ms (10 trials for each prepulse intensity). The percentage of PPI (%PPI) induced by each prepulse intensity was calculated as follows: ((mean startle amplitude on pulse-alone trials – mean startle amplitude on prepulse-plus-pulse trials)/(mean startle amplitude on pulse-alone trial)) × 100%.

Finally, a third block consisting of five startle pulses completed the session. Analysis of startle amplitude on pulse-alone trials across all three blocks served to measure startle habituation. A complete test session lasted 23 min.

Open-field locomotor activity

Locomotor activity was measured similar to previous studies (Jones et al., 2011) using 12 clear Perspex chambers (39.5 cm long × 23.3 cm wide × 24.5 cm deep) with metal grid lids placed in a dimly lit (50–70 lx) chamber. The chambers were placed in frames containing 2 levels of a 4 × 8 photobeam configuration (Photobeam Activity System; San Diego Instruments). Two consecutive breaks of adjacent beams within the lower level of photobeams generated a locomotor count. To start a session, rats were placed into the center of the chambers. Total locomotor counts were calculated for each 10 min block of testing.

Testing the effects of prefrontal muscimol and picrotoxin infusions: experimental design

The experimental design was similar to previous experiments investigating the effects of intracerebral pharmacological manipulations on locomotor activity and on startle/PPI (Bast et al., 2001). Effects of prefrontal muscimol and picrotoxin were tested in separate between-subjects experiments, with the different test chambers and the testing order counterbalanced across groups as far as possible; between-subjects designs were chosen because between-day habituation of startle and locomotor responses and the tendency of PPI to increase across testing days (also see Swerdlow et al., 2000) may confound within-subjects studies. After random allocation to groups, all rats underwent baseline tests before the day of infusion. This allowed us to verify if groups showed comparable baseline values of the sensorimotor measures of interest (and, if not, to reallocate rats to match groups as far as possible). In addition, to verify further that any group differences on the infusion day reflected temporary infusion effects, rather than any other confounding factors, rats were retested on the day after the infusion. Therefore, both the startle/PPI and the locomotor experiments comprised three successive days: day 1 to obtain baseline measures, day 2 to test the effects of the infusions, and day 3 to obtain rebaseline measures.

Startle and PPI

Sixty rats preimplanted with prefrontal guide cannulae were used to examine the effects of prefrontal muscimol (37 rats) and picrotoxin (23 rats) infusion. On day 1, all rats underwent baseline startle and PPI testing without infusion. On day 2 (the day of infusion), rats were tested for startle response and PPI 10 min after the infusion. To examine the effects of prefrontal muscimol infusions, 37 rats received infusions of saline (n = 16), 62.5 ng (n = 7), 125 ng (n = 8), or 250 ng (n = 6)/side muscimol (the reason for the unequal group sizes is explained below, after the next paragraph describing the locomotor experiments). To examine the effects of picrotoxin infusions, another 23 rats received infusions of saline (n = 5), 75 ng (n = 6, but data obtained from only n = 5 due to technical problems), 150 ng (n = 6), or 300 ng (n = 6)/side picrotoxin. On day 3, all rats were retested without a preceding infusion as on day 1 to rule out long-term effects of the infusions.

Locomotor activity

One week later, all rats included in the startle and PPI experiments (except for one rat in the muscimol study that had lost its cannula implant) were used for locomotor studies. On day 1, each rat was placed in one of the test chambers for 1 h to measure baseline locomotor activity. On day 2, infusion effects were tested. Based on previous studies examining the locomotor effects of prefrontal muscimol (Marquis et al., 2007) or of GABA-A antagonists (Matsumoto et al., 2003; Enamoto et al., 2011), we expected muscimol to reduce locomotor activity and picrotoxin to increase it. To test the effects of muscimol, rats were infused with saline (n = 14) or 62.5 ng (n = 7), 125 ng (n = 8), or 250 ng (n = 7)/side muscimol immediately before locomotor activity was tested for 90 min. To test the effects of prefrontal picrotoxin, rats were first placed in the test chamber for 30 min to achieve further habituation of the locomotor response to minimize the possibility that high levels of activity would make it difficult to detect any picrotoxin-induced locomotor hyperactivity. After the 30 min, rats received infusions of saline

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showed similarly depressed locomotor activity in series 1 and 3 (series 1, cortex were mounted on slides and stained with cresyl violet. Placements

Data from the muscimol and picrotoxin studies were analyzed sepa-

Data were analyzed by ANOVA with dose as a within-subjects factor,

ranges (saline and 125 ng muscimol or 150 ng picrotoxin, respectively).

dose ranges were combined for presentation and analysis, with one aver-

For both the muscimol and the picrotoxin studies, data from the two

Statistical analysis of behavioral studies

Verification of cannula placements for behavioral studies

After the completion of the experiments, rats were anesthetized with a

Statistical analysis of behavioral studies

SCSRT data

For both the muscimol and the picrotoxin studies, data from the two
dose ranges were combined for presentation and analysis, with one aver-
age value calculated for the conditions that were included in both dose
ranges (saline and 125 ng muscimol or 150 ng picrotoxin, respectively).

Data were analyzed by ANOVA with dose as a within-subjects factor,
followed by post hoc comparisons using Fisher’s LSD test.

Locomotor and startle/PPI data

Data from the muscimol and picrotoxin studies were analyzed sepa-

Acute in vivo electrophysiology to characterize neural effects of prefrontal drug microinfusions

Implantation of recording array and infusion cannula

Rats were anesthetized using isoflurane delivered in medical air (induc-
tion: 4–5%; maintenance: 1–3%) and were secured in a stereotactic
frame. To minimize the risk of pain, EMLA cream (3%; AstraZeneca) was
used on the ear bars and lidocaine (4% w/v; South Devon Healthcare)
was applied to the incision site on the scalp. Throughout the experiment,
rectal temperature was maintained at ~37°C using a heating pad con-
trolled by a rectal probe. After scalp incision, bregma and lambda were
aligned horizontally and the bone was removed over the medial prefron-
tal cortex. The exposed dura was incised and the underlying cortex kept
moist throughout the experiment with 0.9% saline.

A custom-made infusion-recording assembly (cf. Fig. 6A) was then
implanted into the right prefrontal cortex. The assembly consisted of a 33
gauge stainless steel infusion cannula attached to an eight-channel micro-
wire array (eight 50 μm Teflon-coated stainless steel wires with an
impedance of ~100 kΩ measured at 1 kHz; Robinson, 1968; and ar-
anged in one row spanning ~2 mm) with a stainless steel ground wire
(NB Labs); the array was connected via a head stage to the recording
system. The cannula tip about touched the electrodes and was positioned
~0.5 mm above the tips of the central electrodes (number 3 to 4). The
end of the cannula was connected to a 1 μl Hamilton syringe via Teflon
tubing (0.65 mm outer and 0.12 mm inner diameter; Bioanalytical Sys-
tems). Infusion cannula and tubing were filled with drug solution or
saline (made up as described for the behavioral studies). A small air
bubble was trapped where the tubing was connected to the syringe and
movement of the bubble served to indicate a successful infusion. To
prevent leakage and drug diffusion before the infusion, the piston of the
syringe was pulled back to draw up a 0.25 μl air “plug” (similar to St
Peters et al., 2011) before the infusion-recording assembly was inserted
into the brain (except for the first nine experiments; subsequent inspec-
tion of the data did not indicate a difference between experiments with
and without air plug). The assembly was fixed to the arm of the stereo-
taxic frame such that the microwire array was arranged parallel to the
midline of the brain and between the midline and infusion cannulae. The
assembly was slowly lowered toward the target position in the medial
prefrontal cortex, with the cannula tip aimed at the same coordinates as
the infusion cannulae in the behavioral experiments: 3 mm anterior and
0.6 mm lateral from bregma and 3.5 mm ventral from dura. Positioning
of the infusion-recording assembly was followed by a period of stabiliza-
tion (at least 30 min), during which anesthesia was adjusted to a stable
level (to maintain a stable breathing rate of ~50–60/min) that would be
maintained during baseline and postinfusion recordings.

Multunit and LFP recordings

To record extracellular measures of neural activity, the electrode array
was connected via a unity-gain multichannel head stage to a multichan-
nel preamplifier (Plexon), which amplified (1000×) the analog signal
and band-pass filtered it into multunit spikes (250 to 8 kHz) and LFP
signals (0.7 to 170 Hz). Recordings were made against ground, with the
ground wire of the electrode array clamped to the ear bars using a croc-
odile clip and a lead linking the stereotaxic frame to the ground jack
on the amplifier. The analog signals were fed to a multichannel acquisition
processor system (Plexon), which provided additional computer-
controllable amplification (final gain up to 32,000), additional filtering
of multunit data (500 to 5 kHz), and digitization of spikes at 40 kHz
(providing 25 μs precision on each channel at 12 bit resolution) and of
LFP data at 1 kHz. Multunit data were also displayed on an analog-
digital oscilloscope and monitored using a loudspeaker. Multunit and
LFP data were viewed online with Real-Time Acquisition System Pro-
grams for Unit Timing in Neuroscience (RASPUTION) software (Plexon).
Using RASPUTTON, neural activity data were recorded for a 30 min base-
line period and a 90 min postinfusion period. LFP data were recorded
continuously and multunit spikes were recorded when a predefined
amplitude threshold of ~240 μV was exceeded (visual inspection of the
oscilloscope trace indicated that this threshold corresponded to ~2 ×
average negative signal deflection per time, or more, outside threshold-
crossing spikes; compare multunit recording traces in Fig. 7). Examples
of continuous multi-unit traces for presentation purposes, as shown in Figure 7, were recorded using a DATAQ Instruments AD interface (Model DI149 HS).

Microinfusion procedure and experimental design
After 30 min of baseline recordings, the piston of the 1 µL syringe was moved manually at a slow speed (infusion speed of ~0.5 µL/min as in the behavioral studies) to remove the 0.25 µL air plug from the injector tip and to inject 0.5 µL of saline or of drug solution into the medial prefrontal cortex. To verify that liquid was successfully infused into the brain, we monitored movement of the air bubble that was trapped where the infusion tubing and syringe were connected. The start and end times of the infusion were recorded so that preinfusion and postinfusion periods could accurately be identified for the subsequent data analysis. Visual inspection of LFP traces and multiunit spike waveforms did not indicate infusion-induced electrical artifacts (also compare Fig. 7B). After completion of the infusion, recordings continued for at least 60 min.

The effects of saline and drug infusions on the time course of multiunit and LFP data were determined between subjects. The main focus was on comparing and contrasting the effects of the highest dose of muscimol (250 ng) and picrotoxin (300 ng). We started out testing the effects of these 2 infusions (n = 6 in each group). Our initial experiments revealed very pronounced, largely opposite, effects of the two drugs, with the picrotoxin effects reliably detectable by visual inspection of the LFP traces (see Results section). At this point, it emerged from our behavioral experiments that 150 ng of picrotoxin had somewhat distinct behavioral effects from the 300 ng dose. For this reason, we decided to include a group of rats to assess the electrophysiological effects of 150 ng of picrotoxin (n = 6). In addition, we included a few rats receiving saline infusions (n = 4) to rule out nonspecific infusion effects, which may confound the interpretation of the drug effects. Moreover, we added a few experiments with 300 ng picrotoxin as a positive control (an additional 4 rats, bringing the total in the 300 ng group to n = 10).

Verification of electrode placements
At the end of each experiment, current (1 mA, 10 s) was passed through two pairs of the stainless steel microwires of the array to deposit ferric ions at the tip of the positive electrode and to mark its position. At least two electrode positions were marked for each experiment, one at each end of the array. The infusion-recording assembly was then removed and the rat killed by an overdose of anesthetic. Brains were removed and stored in a 4% paraformaldehyde solution with 4% potassium ferrocyanide for at least 2 days before 80 µm coronal sections were prepared on a vibratome. Iron deposits at the electrode tips were revealed by the Prussian blue reaction. Locations of the marked electrode tips were determined using a light microscope and mapped onto coronal sections of a rat brain stereotaxic atlas (Paxinos and Watson, 1998). In some rats, some of the electrodes were located between hemispheres or within the anterior forceps of the corpus callosum; the data from these channels were excluded from the analysis.

Analysis of electrophysiological data
NeuroExplorer version 4 (Nex Technologies) was used to calculate various parameters from the multiunit data (firing rate and burst parameters) and LFP data (power spectral densities). These parameters were calculated for the data from each electrode for each 5 min bin of the baseline and postinfusion recording periods. For normalization to baseline, values obtained from the individual channels were divided by the average values obtained from the same channel during the 6 (5 min) baseline blocks. Values were averaged across all channels per individual rat, and these average values were used to calculate means for the different infusion groups. All data are presented as mean ± SEM. Using ANOVA with infusion group as the between-subjects factor and 5 min block as the within-subjects factor, the data were examined for significant differences between infusion groups. Fisher’s LSD test was used for post hoc comparisons. The accepted level of significance was p < 0.05.

Multiunit data and burst analysis
In addition to overall firing rates, we measured and analyzed parameters characterizing firing during bursts, periods of relatively high spiking that are separated by periods of comparatively little spiking (Legény and Salcman, 1985; Lisman, 1997; Cooper, 2002; Izhikevich et al., 2003). We analyzed bursts for three reasons. First, in neocortical recordings under anesthesia, the burst periods resemble the continuously activated neural network state during wakefulness (Destexhe et al., 2007; Haider and McCormick, 2009), whereas the intermittent periods with little firing show limited similarity to wake recordings. Second, in our prefrontal recordings under anesthesia, we often find that overall neocortical firing rates show pronounced variations across time, reflecting alternations between time stretches with much and little bursting (accompanied by higher-amplitude and lower-amplitude, respectively, LFP signal; compare Clement et al., 2008), whereas within-burst firing rates are quite stable across time, providing a stable baseline. (The alternations between periods of much and little bursting may be due to variations in the level of anesthesia, even though we kept the delivery rate of anesthetic during recording constant and the breathing rate was stable.) Third, bursts have been suggested to be key units of neural information processing, increasing the reliability and/or selectivity of neural communication (Lisman, 1997; Cooper, 2002; Izhikevich et al., 2003; Larkum, 2013). Similar to previous studies (Homayoun et al., 2005; Stevenson et al., 2007), we detected prefrontal bursts using the Poisson surprise method (Legény and Salcman, 1985), as implemented in NeuroExplorer version 4. The Poisson surprise method is well suited to detect bursts with irregular spike patterns as observed in prefrontal cortex (Homayoun et al., 2005; Stevenson et al., 2007). Bursts are defined as spike trains with relatively high firing rate, which are surprising (i.e., improbable) based on the average spike rate during the rest of the analysis window. A burst is characterized by its surprise value S, which is the negative natural logarithm of the probability that the relatively high burst firing rate is merely a chance occurrence within a random spike pattern. Based on a previous study (Stevenson et al., 2007), our final analysis included bursts with surprise values of S > 3 (this means that there is an approximate probability of 0.05 for similar spike patterns to occur by chance as part of a random spike train). However, it is important to note that a preliminary analysis using S > 10 yielded very similar results, indicating that the key findings are largely independent of the exact surprise value chosen. The following burst parameters were calculated for each 5 min block: number

![Figure 1](https://example.com/figure1.png)
of bursts, percentage of spikes fired as bursts, mean firing rate within bursts, mean burst duration, and interburst interval. Before calculating average values for burst duration and within-burst firing rate, channels that did not record bursts during all 5 min blocks (i.e., those that recorded 0 bursts during at least 1 5 min block) were excluded; before calculating average interburst intervals, channels that did not record more than 1 burst during all 5 min blocks were excluded (for 1 rat that received a muscimol infusion, there was not one electrode that recorded more than 1 burst in all 5 min blocks; therefore, this rat had to be excluded from the analysis of interburst intervals, so that only 5 rats remained in the muscimol group for this analysis).

**LFP data**
Using the Powerspectral Density analysis in Neuroexplorer version 4, which applies fast Fourier transform (FFT) analysis to the LFP signal, we calculated the area under the curve (AUC) of the power spectral density function (PSD) from 0.7–170 Hz as a measure of overall LFP power for every 5 min block of the preinfusion and postinfusion recording periods (similar to Lodge, 2011). Although FFT analysis of nonstationary signals, especially over long time blocks, may cause frequency leakage, the smearing of power from the true frequency to adjacent frequencies is not a problem if, as in the present study, the analysis is aimed at assessing infusion-induced changes in overall LFP power across broad frequency ranges, rather than changes in frequency-specific power.

**Results**
**Infusion cannula placements in behavioral studies**
All infusion cannula tips were placed within the medial prefrontal cortex within an area that corresponded approximately to 2.7–

<table>
<thead>
<tr>
<th>Experiment, doses</th>
<th>Correct latency (s)</th>
<th>Collect latency (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscimol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.49 ± 0.03</td>
<td>1.46 ± 0.11</td>
</tr>
<tr>
<td>62.5 ng</td>
<td>0.56 ± 0.08</td>
<td>1.73 ± 0.30</td>
</tr>
<tr>
<td>125 ng</td>
<td>0.71 ± 0.12</td>
<td>2.11 ± 0.36</td>
</tr>
<tr>
<td>250 ng</td>
<td>1.08 ± 0.29</td>
<td>2.37 ± 0.75</td>
</tr>
<tr>
<td>Picrotoxin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.95 ± 0.13</td>
<td>1.58 ± 0.21</td>
</tr>
<tr>
<td>75 ng</td>
<td>0.62 ± 0.07</td>
<td>1.73 ± 0.30</td>
</tr>
<tr>
<td>150 ng</td>
<td>1.10 ± 0.13</td>
<td>2.53 ± 0.55</td>
</tr>
<tr>
<td>300 ng</td>
<td>1.84 ± 0.50</td>
<td>1.86 ± 0.51</td>
</tr>
</tbody>
</table>

*Significantly different from saline and 62.5 ng.

*Significantly different from saline and both lower doses.
was reduced at all muscimol doses compared with saline ($F_{(3,33)} = 10.68, p < 0.0001$; post hoc tests, all $p < 0.003$). At 250 ng, accuracy was lower than at 62.5 ng ($p < 0.04$), with no further differences between doses (all $p > 0.15$). In parallel, the percentage of omissions was increased by all doses compared with saline ($F_{(3,33)} = 10.54, p < 0.0001$; post hoc tests, all $p < 0.0004$), with no difference between doses (all $p > 0.25$). In addition, premature responses were increased at all doses compared with saline ($F_{(3,33)} = 5.42, p < 0.004$; post hoc tests, all $p < 0.04$), with no differences between doses (all $p > 0.21$).

Perseverative responses were not significantly affected by prefrontal muscimol infusions ($F_{(3,33)} = 1.26, p = 0.30$), even though there was a numerical increase in perseverative responses with increasing muscimol dose. As would be expected as a consequence of the rats obtaining less reinforcement due to their impaired task performance, the total number of completed trials was decreased at all doses compared with saline ($F_{(3,33)} = 11.54, p < 0.0001$; post hoc tests, all $p < 0.0002$), with no differences between doses (all $p > 0.49$). In addition, correct-response latencies were dose dependently increased ($F_{(3,33)} = 3.99, p < 0.02$). At 250 ng, muscimol increased or tended to increase correct response latencies compared with saline and 62.5 ng ($p < 0.009$) and compared with 125 ng ($p = 0.056$), with no further differences between doses ($p > 0.24$). In contrast, reward collection latencies were unaffected ($F_{(3,33)} = 1.03, p = 0.39$; Table 2), indicating that muscimol did not induce gross motivational or motor impairments.

### Prefrontal picrotoxin causes attentional deficits

Picrotoxin infusions (75, 150, or 300 ng/side) caused dose-dependent attentional deficits, as indicated by decreased accuracy and increased omissions at the higher doses, whereas response control was unaffected (Fig. 3). Accuracy was dose dependently reduced ($F_{(3,33)} = 12.22, p < 0.0001$), with 300 ng reducing accuracy compared with saline and the other two doses ($p < 0.002$) and 150 ng tending to reduce accuracy compared with saline ($p = 0.075$) and to 75 ng ($p = 0.10$), whereas 75 ng did not affect accuracy compared with saline ($p = 0.90$). Omissions were similarly increased by 300 and 150 ng ($F_{(3,33)} = 8.70, p < 0.0003$) compared with saline (both $p < 0.004$) and 75 ng (300 ng, $p < 0.002$; 150 ng, $p = 0.09$), with no difference between these two doses ($p = 0.12$). At 75 ng, omissions did not differ from saline ($p = 0.16$). Picrotoxin infusions did not affect measures of response control, with both premature and perseverative responses unchanged (both $F_{(3,33)} < 1$). Consistent with the poor task performance and hence reduced reinforcement, picrotoxin infusions dose dependently reduced the number of trials initiated by the rats ($F_{(3,33)} = 9.56, p < 0.0002$). The number of trials was decreased at 300 ng compared with saline and the two other doses (all $p < 0.02$). At 150 ng, picrotoxin tended to reduce the number of trials compared with saline ($p = 0.07$) and 75 ng ($p < 0.05$), which did not differ from each other ($p = 0.87$). Latencies to make a correct response were dose dependently increased by 300 ng ($F_{(3,33)} = 4.54, p < 0.008$) compared with saline and the other two doses (all $p < 0.04$), with no further differences (all $p > 0.16$). In contrast, latencies to collect the reward were not affected by picrotoxin infusions ($F_{(3,33)} = 1.52, p = 0.23$; Table 2), indicating that picrotoxin did not induce gross motivational or motor impairments.

### Sensorimotor effects

Prefrontal muscimol dose dependently reduces, whereas picrotoxin increases, locomotor activity at the highest dose Muscimol. During the 90 min open-field test after prefrontal infusion of saline or muscimol (62.5, 125, or 250 ng/side), locomotor activity, measured as consecutive photobeam breaks, was dose dependently decreased ($F_{(3,32)} = 4.29, p < 0.02$) across the whole session (interaction infusion $\times$ 10 min bin: $F_{(24,256)} < 1$; Fig. 4A). Post hoc comparisons revealed that, compared with the saline infusions, locomotor activity was significantly reduced by muscimol infusions at 125 ng/side ($p < 0.02$) and 250 ng/side ($p < 0.004$), but not at 62.5 ng/side ($p = 0.25$); there was also a trend for activity in the 250 ng group to be lower than in the 62.5 ng group ($p = 0.10$), but no further group differences ($p > 0.21$).

Picrotoxin. In the 30 min before infusion of saline or picrotoxin (75, 150, or 300 ng/side), the different infusion groups showed similar locomotor activity (main effect or interaction involving the factor infusion: $F < 1.84, p > 0.12$). Prefrontal picrotoxin caused a dose-dependent increase in locomotor activity starting ~10 min after the infusions and lasting for ~20 min (main effect of infusion: $F_{(3,19)} = 5.58, p < 0.007$; interaction infusion $\times$ 10 min bin: $F_{(15,95)} = 2.61, p < 0.003$; Fig. 4B). Separate ANOVAs and post hoc comparisons of locomotor activity during the nine 10 min bins after the infusions indicated that, compared with saline and the lower picrotoxin doses, 300 ng picrotoxin increased locomotor activity during the second and third 10 min bin after infusion ($F > 4.00, p < 0.03$; post hoc tests, $p < 0.01$), whereas groups did not differ during the other 10 min
Startle and PPI: no clear effects of prefrontal muscimol or picrotoxin

Muscimol. Prefrontal muscimol infusions (62.5, 125, or 250 ng/side) did not substantially affect startle and PPI measures compared with saline infusions. Even though statistical analysis revealed subtle group differences on the infusion day, these differences were unrelated to the drug infusions and reflected pre-existing differences that were already present in the prospective infusion groups during baseline testing on the day before the infusion (despite our attempt to match groups; Fig. 5A). Startle responses on pulse-alone trials were similar for all groups during the test sessions after the prefrontal infusions, even though rats infused with 62.5 ng muscimol tended to show lower startle amplitudes (Fig. 5A, left). This was reflected by a main effect of group on baseline startle responses during pulse-alone trials across the three test blocks ($F_{(3,33)} = 3.06, p < 0.05$; no interaction group $\times$ test block, $F < 1$). Importantly, rats in the prospective 62.5 ng muscimol group already tended to show lower startle amplitude during baseline testing 1 d before the infusions (Fig. 5A, left, inset), with ANOVA revealing a trend for a main effect of prospective infusion group on baseline startle measures across test blocks ($F_{(3,33)} = 2.52, p = 0.08$) and a significant interaction group $\times$ test block ($F_{(6,66)} = 2.47, p < 0.04$), which reflected that differences were most pronounced during the first block of 10 pulse-alone trials before habituation of the startle response. PPI at the two higher prepulse intensities (80 and 84 dB) was similarly strong for all groups during the test sessions on the day of infusion, whereas there was overall little PPI at the 72 and 76 dB prepulses, as is often observed in Lister hooded rats (Weiss et al., 2000; Jones et al., 2011); at one of the lower prepulse intensities (76 dB), the group receiving 250 ng/side tended to show lower PPI than the other groups (Fig. 5A, right). These observations were reflected by a highly significant effect of prepulse intensity ($F_{(3,99)} = 93.13, p < 0.0001$) and a significant interaction between prepulse intensity and infusion groups ($F_{(9,99)} = 2.56, p < 0.02$). Importantly, the prospective 250 ng muscimol group already showed reduced PPI at the lower prepulse intensities during baseline measurements on the day before the infusions (Fig. 5A, right, inset), with ANOVA of baseline PPI measures showing a significant interaction of prospective infusion group and prepulse intensity ($F_{(9,99)} = 2.04, p < 0.05$). Therefore, overall, prefrontal muscimol infusions did not substantially affect startle and PPI measures.

Picrotoxin. During testing after infusion of saline or picrotoxin (75, 150, or 300 ng/side), all infusion groups showed similar startle measures on pulse-alone trials across test blocks and similar PPI (Fig. 5B). ANOVA did not reveal any significant main effect or interaction involving infusion group (all $F < 1.7, p > 0.16$). The absence of an effect of prefrontal picrotoxin on PPI contrasts with a previous report that PPI was more than halved by prefrontal infusion of a comparably low dose of picrotoxin (10 ng/0.5 $\mu$L/side) in Sprague Dawley rats (Japha and Koch, 1999). Although a variety of procedural variables may affect the outcome of PPI studies (Swerdlow et al., 2000), the discrepant findings concerning the effects of prefrontal picrotoxin most likely reflect strain differences. First, Sprague Dawley rats may be particularly sensitive to intracerebral picro-
toxin. Consistent with this, Japha and Koch (1999) also reported a strong trend for ventral hippocampal infusions of 5–10 ng/side to disrupt PPI in Sprague Dawley rats, with higher doses causing seizures, whereas we did not observe any behavioral effects after ventral hippocampal infusions of such low doses in Wistar rats, with locomotor hyperactivity and PPI disruption only emerging at 100–150 ng and no evidence for seizures (Bast et al., 2001). Second, the pharmacological modulation of PPI is highly strain dependent (Swerdlow et al., 2008). In fact, our present finding that prefrontal picrotoxin does not disrupt PPI in Lister hooded rats, contrasting with the marked PPI disruption by prefrontal picrotoxin in Sprague Dawley rats (Japha and Koch, 1999), converges with other findings suggesting that PPI in Lister hooded rats may be less sensitive to pharmacological disruption. For example, Lister hooded rats are less sensitive to PPI disruption by apomorphine than Wistar and Sprague Dawley rats (Weiss et al., 2000) and, whereas ventral hippocampal picrotoxin disrupts PPI in Wistar (Bast et al., 2001) and Sprague Dawley (Japha and Koch, 1999) rats (albeit at different doses), we did not find this effect in Lister hooded rats (Bast et al., 2010).

In vivo electrophysiology

Electrode placements

All electrodes from which data were included in the analysis were placed within an area of the prefrontal cortex that corresponded approximately to 2.2–4.2 mm anterior to bregma in the atlas by Paxinos and Watson (1998), with the central electrodes of the array and the infusion cannula placed within the medial prelimbic area (Fig. 6).

Qualitative observations: picrotoxin causes LFP spike-wave discharges and intensifies the multunit burst-firing pattern within the prefrontal cortex

Prefrontal recordings under isoflurane anesthesia were characterized by “slow oscillations” of neural activity; that is, alterations between relatively silent and relatively active periods at frequencies of <1 Hz, as is typical for neocortical recordings during sleep and anesthesia (Steriade et al., 1993; Castro-Alamancos, 2000; Isomura et al., 2006). The active periods showed comparatively higher-amplitude LFP signals and multunit burst firing, whereas the silent periods showed little LFP signal and little unit firing (Fig. 7A). Prefrontal picrotoxin infusion intensified this pattern, causing large-amplitude LFP patterns consisting of a sharp negative deflection followed by a positive wave and more intense bursts (Fig. 7B). This effect could be detected by mere visual inspection of the neural activity data in all 10 experiments involving infusion of 300 ng picrotoxin and also in most experiments (four of six) involving infusion of 150 ng, whereas no such effect was observed after muscimol or saline infusion. Similar LFP patterns, also referred to as “spike-wave” discharges (with “spike” referring to a sharp negative LFP deflection, not a unit spike), have been documented by previous studies using in vivo LFP recordings under anesthesia to examine the effects of local application of GABA-A antagonists in neocortex (Neckelmann et al., 1998; Castro-Alamancos, 2000), including medial prefrontal cortex (Lodge, 2011). Consistent with the absence of behavioral seizure signs at the picrotoxin doses used in the present study, our in vivo recordings did not reveal the LFP characteristics of seizures induced by local GABA-A antagonists, namely, a fast sequence of sharp negative deflections (so-called LFP spikes, 10–15 Hz) superimposed on spike-wave discharges (Neckelmann et al., 1998; Steriade and Contreras, 1998; Castro-Alamancos, 2000; Bragin et al., 2009).

Multunit data: muscimol inhibits and picrotoxin disinhibits neuron firing within bursts, and picrotoxin (300 ng) increases the proportion of spikes fired in bursts

Among the multunit parameters examined, within-burst firing rates and the proportion of spikes fired as part of bursts showed the clearest infusion-induced changes (Fig. 8), with these measures showing good baseline stability and consistent drug effects across experiments.

Within-burst firing rates were decreased by muscimol (250 ng) infusion and increased similarly by infusion of the two doses of picrotoxin (150 or 300 ng), whereas saline infusion had no effect (Fig. 8A). ANOVA of normalized within-burst firing rates across the 6 5 min blocks before infusion and the 12 5 min blocks after infusion revealed a significant interaction of infusion group and 5 min block ($F_{(17,374)} = 3.62, p < 0.0001$). This interaction reflected that firing rates decreased after muscimol infusion ($F_{(17,85)} = 4.97, p < 0.0001$) and increased after infusion of picrotoxin, at both 150 ng ($F_{(17,85)} = 4.53, p < 0.0001$) and 300 ng ($F_{(17,153)} = 5.83; p < 0.0001$), whereas firing rates remained relatively stable across time in the experiments involving saline infusion ($F_{(17,51)} < 1$). The proportion of spikes fired in burst was increased by picrotoxin (300 ng), whereas muscimol tended to decrease this measure (Fig. 8B). ANOVA of the normalized percentage of spikes fired within bursts across the 6 5 min blocks before infusion and the 12 5 min blocks after infusion revealed a significant interaction of infusion group and 5 min block ($F_{(17,374)} = 1.86, p < 0.001$). This interaction reflected that the percentage of spikes fired within bursts tended to decrease after muscimol infusion ($F_{(17,85)} = 1.55, p = 0.098$) and increased after infusion of 300 ng picrotoxin ($F_{(17,153)} = 3.83; p < 0.0001$),
for related time in the experiments involving infusion of 150 ng picrotoxin whereas the percentage of spikes remained relatively stable across the predefined spike-detection threshold (picrotoxin). The two stippled horizontal lines in the multiunit traces indicate the zero-voltage line and (bottom, electrodes 1, 3, 6, and 8) from one exemplary experiment involving infusion of 300 ng picrotoxin. The two stippled horizontal lines in the multiunit traces indicate the zero-voltage line and (bottom, electrodes 1, 3, 6, and 8) from one exemplary experiment involving infusion of 300 ng picrotoxin or saline (both F < 1.4, p > 0.17). Picrotoxin’s different effects on overall firing rates compared with within-burst firing rates (compare Fig. 8A), especially at 300 ng, may partly reflect limited baseline stability of overall firing, making detection of drug-induced alterations difficult. In addition, although it increased within-burst firing, 300 ng picrotoxin reduced firing outside bursts (as reflected by an increased percentage of spikes fired in bursts, see Fig. 8B), which may account for the relatively stable overall firing despite increased within-burst firing (compare Sanchez-Vives et al., 2010 for related in vitro findings).

There were also infusion effects on additional burst parameters. First, both 150 and 300 ng picrotoxin increased the number of bursts, whereas muscimol decreased the number (Fig. 9B). ANOVA of normalized numbers of bursts per 5 min block across the 6 5 min blocks before infusion and the 12 5 min blocks after infusion revealed a significant interaction of infusion group and 5 min block (F(51,374) = 1.57, p < 0.05). This interaction reflected that the number of bursts decreased after infusion of muscimol (F(17,51) = 3.65, p < 0.0001) and increased after infusion of picrotoxin at both 150 ng (F(17,51) = 1.77, p < 0.05) and 300 ng (F(17,153) = 1.96; p < 0.02), whereas the number of bursts did not show a consistent change across time in the experiments involving saline infusion (F(17,51) < 1) even though the measure showed substantial variability. Second, drug infusions altered the temporal pattern of bursting, as reflected by changes in burst duration and interburst interval, even though interburst intervals in particular showed high variability (Fig. 9C,D). Muscimol increased burst duration, but had no significant effect on interburst interval. Picrotoxin appeared to affect both measures dose dependently: 150 ng decreased both measures, whereas 300 ng caused a short-lasting increase (15–20 min), after which measures decreased similar to what was seen with 150 ng, possibly reflecting that amounts of picrotoxin in the vicinity of the infusion site decreased due to diffusion. ANOVA of normalized burst duration across the 6 5 min blocks before infusion and the 12 5 min blocks after infusion revealed a significant interaction of infusion group and 5 min block (F(51,374) = 2.30, p < 0.0001). This interaction reflected that burst duration increased after muscimol infusion (F(17,51) = 2.38, p < 0.005) and decreased after 150 ng picrotoxin (F(17,51) = 3.45, p < 0.001), but increased and then decreased at 300 ng (F(17,153) = 3.41; p < 0.0001); burst duration did not show clear changes across time in the experiments involving saline infusion (F(17,51) < 1). ANOVA of normalized interburst intervals across the 6 5 min blocks before infusion and the 12 5 min blocks after infusion revealed a significant interaction of infusion group and 5 min block (F(51,374) = 1.75, p < 0.002). This interaction reflected that interburst intervals decreased across time in the 150 ng picrotoxin group (F(17,51) = 2.14, p < 0.02), whereas they increased and then decreased after infusion of 300 ng picrotoxin (F(17,153) = 2.14, p < 0.02); in addition, interburst

whereas the percentage of spikes remained relatively stable across time in the experiments involving infusion of 150 ng picrotoxin (F(17,51) < 1) or saline (F(17,51) < 1).

Additional effects
Infusions affected additional measures of multiunit firing, even though these effects were less clear due to limited baseline stabil-

Figure 7. LFP spike-wave discharges and intensified multiunit burst-firing pattern within prefrontal cortex after picrotoxin infusion. Multiunit recording traces (top, electrode 8) and LFP traces (bottom, electrodes 1, 3, 6, and 8) from one exemplary experiment involving infusion of 300 ng picrotoxin. The two stippled horizontal lines in the multiunit traces indicate the zero-voltage line and the predefined spike-detection threshold (−240 μV). A, Baseline recording −5 min before infusion. B, Recordings −15 min after picrotoxin infusion. Note the compressed, intensified burst periods in 15 min after picrotoxin infusion. Note the compressed, intensified burst periods in 15 min after picrotoxin infusion.
intervals seemed to increase slowly across the postinfusion period in the muscimol group, even though this effect was far from significant ($F_{(17,68)} = 1.20, p = 0.29$), whereas the interburst interval did not show consistent changes from baseline across time after saline infusions ($F_{(17,51)} < 1$).

**Picrotoxin increases prefrontal LFP power**
Picrotoxin markedly increased overall LFP power, consistent with the marked spike-wave discharges detectable by mere visual inspection, whereas muscimol did not significantly affect power (Fig. 10). ANOVA of the normalized AUC of PSD across the 6 5-minute blocks showed a significant difference between groups ($F_{(17,68)} = 1.20, p = 0.29$).
min blocks before infusion and the 12.5 min blocks after infusion revealed a significant interaction of infusion group and 5 min block \((F_{(51,174)} = 2.53, \ p < 0.0001)\). This interaction reflected that, overall, LFP power markedly increased after infusion of picrotoxin at both 150 ng \((F_{(7,180)} = 3.09, \ p < 0.0005)\) and 300 ng \((F_{(17,153)} = 7.77, \ p < 0.0001)\), with the peak of the effect at 150 ng being somewhat smaller and delayed by ~10 min compared to 300 ng. In contrast, muscimol \((F_{(17,45)} = 1.15, \ p = 0.33)\) and saline infusion \((F_{(17,51)} < 1)\) had no significant effects.

### Mean baseline values of electrophysiological parameters
The mean baseline values of most of the electrophysiological parameters analyzed showed no difference between infusion groups. All infusion groups showed similar mean baseline firing rates, both with respect to overall firing (in spikes/s, saline = 17.5 ± 5.9; 250 ng muscimol, 10.5 ± 5.4; 150 ng picrotoxin, 15.6 ± 4.3; 300 ng picrotoxin, 39.1 ± 14.3; \(F_{(3,22)} = 1.44, \ p = 0.26)\) and with respect to within-burst firing (in spikes/s, saline, 223.3 ± 33.3; 250 ng muscimol, 106.9 ± 29.3; 150 ng picrotoxin, 218.5 ± 32.0; 300 ng picrotoxin, 199.9 ± 34.6), even though there was a trend for the latter to be reduced in the muscimol group compared with the other 3 groups \((F_{(3,22)} = 2.34, \ p = 0.10)\). Similarly, the baseline number of bursts per 5 min bin did not significantly differ between groups (saline, 132.4 ± 57.7; 250 ng muscimol, 42.4 ± 11.7; 150 ng picrotoxin, 117.4 ± 38.6; 300 ng picrotoxin, 236.6 ± 67.8; \(F_{(3,22)} = 2.22, \ p = 0.11\)). Moreover, the temporal pattern of burst firing was similar across all infusion groups, with all groups showing similar mean baseline burst duration (saline, 0.23 ± 0.03 s; 250 ng muscimol, 0.48 ± 0.21 s; 150 ng picrotoxin, 0.23 ± 0.03 s; 300 ng picrotoxin, 0.19 ± 0.04 s; \(F_{(3,22)} = 1.79, \ p = 0.18)\) and mean baseline interburst intervals (saline, 6.8 ± 2.3 s; 250 ng muscimol, 8.0 ± 2.6 s; 150 ng picrotoxin, 5.8 ± 2.0 s; 300 ng picrotoxin, 5.9 ± 2.1 s; \(F_{(3,22)} < 1)\). Surprisingly, the mean baseline values of some electrophysiological parameters showed differences between infusion groups. ANOVA of the percentage of spikes fired within bursts revealed a main effect of group \((F_{(3,22)} = 6.60, \ p < 0.003)\). The percentage of spikes fired in bursts (saline, 69.3 ± 4.1; 250 ng muscimol, 28.6 ± 6.4; 150 ng picrotoxin, 72.3 ± 4.6; 300 ng picrotoxin, 52.8 ± 7.8) was significantly reduced in the muscimol group compared with all other 3 groups (all \(p < 0.02)\) and tended to be lower in the 300 ng compared with the 150 ng picrotoxin group \((p = 0.054)\), whereas neither of the picrotoxin groups differed from the saline group (both \(p > 0.13)\). Finally, ANOVA of mean baseline LFP power, measured as AUC of PSD, revealed a trend for a main effect of infusion group \((F_{(3,22)} = 2.82, \ p = 0.06)\), reflecting reduced values in the muscimol group \((0.006 ± 0.002 \mu V^2)\) and increased values in the 300 ng picrotoxin group \((0.024 ± 0.006 \mu V^2)\), whereas values in the saline group \((0.015 ± 0.004 \mu V^2)\) and the 150 ng picrotoxin group \((0.013 ± 0.002 \mu V^2)\) were similar and intermediate to the other two groups.

To prevent leakage of drug solution into the brain, we used an “air plug” at the injector tip to separate the infusion liquid from the brain tissue before infusion. Indeed, there were no significant differences in the baseline values of most electrophysiological parameters. Moreover, the significant baseline differences that were found between groups (percentage of spikes fired in bursts, LFP power) are not entirely consistent with the infusion effects on the same parameters (e.g., picrotoxin infusions increased percentage of spikes fired in bursts and LFP power compared with saline infusions, whereas mean baseline values of these parameters did not differ between the picrotoxin and saline groups). Therefore, even though leakage of drug from the indwelling injector cannot be ruled out completely, interindividual variability unrelated to drug leakage may have contributed to group differences in baseline values.

### Discussion
Prefrontal microinfusion of muscimol or picrotoxin both caused attentional deficits on the 5CSRT task, whereas only muscimol also impaired response control (increased premature responses). Prefrontal muscimol dose dependently reduced locomotor activity, whereas picrotoxin caused locomotor hyperactivity at the highest dose. Neither muscimol nor picrotoxin affected startle or PPI. *In vivo* electrophysiology revealed that muscimol inhibited prefrontal firing rate, both overall and within bursts (by 30–50% in the 90 min after infusion), whereas picrotoxin particularly increased within-burst firing rate (by 30–70%). In addition, muscimol reduced bursting, whereas picrotoxin enhanced it, and both drugs altered the temporal features of burst firing. Picrotoxin also markedly enhanced prefrontal LFP power.

**Both prefrontal hypoactivation and disinhibition cause attentional deficits and hypoactivation also impairs response control**
Prefrontal muscimol impaired attention and response control (increased premature responses) similar to neurotoxic lesions (Chudasama and Muir, 2001; Passetti et al., 2002; Pezze et al., 2009; Chudasama et al., 2012). Our findings reveal that attention and response control require prefrontal activation. This was not a foregone conclusion: indeed, attentional deficits after prefrontal lesions were suggested to reflect lesion-induced compensatory changes in prefrontal projection sites based on the recent finding that prefrontal muscimol only increased premature responses without affecting attention (Paine et al., 2011). In light of our new data, the absence of attentional deficits may reflect low muscimol doses (6.25–50 ng vs 62.5–250 ng in our study) or low attentional demands (1 vs 0.5 s SD). Murphy et al. (2012), using a higher muscimol dose (500 ng), reported both response control and attentional deficits after prefrontal infusions, consistent with our new data, although increased reward collection latencies indicated that the high dose may have caused nonspecific impairments.

Prefrontal disinhibition caused selective attentional deficits. Consistent with this, Paine et al. (2011) reported attentional deficits on the 5CSRT test after prefrontal infusion of the GABA-A antagonist bicuculline, although increased reward collection latencies indicated that bicuculline may have caused nonspecific impairments. Another study reported attentional deficits on the three-choice serial reaction time test after infusions of the
GABA-A antagonist SR95531 into the most dorsal (anterior cingulate) medial prefrontal cortex (Pehrson et al., 2013). In contrast, in a study targeting more ventral parts (infralimbic infusion sites), bicuculline did not affect attention, but reduced premature responses (Murphy et al., 2012), consistent with the view that ventral prefrontal regions are more important for response control, whereas dorsal regions are more important for attention, a view that has emerged from studies using neurotoxic lesions (Chudasama and Muir, 2001; Passetti et al., 2002; Dalley et al., 2004; Chudasama and Robbins, 2006; Pezze et al., 2009; Chudasama et al., 2012).

Our findings show that sustained attention is highly susceptible to changes in prefrontal neuronal activation, with both reduced and disinhibited activity causing deficits. This resembles the finding that both reduced and increased prefrontal D1 receptor stimulation disrupt attention (Granon et al., 2000). Given that D1 stimulation increases the excitability of prefrontal inhibitory interneurons (Seamans et al., 2001; Tseng and O’Donnell, 2007), decreased D1 stimulation may resemble prefrontal disinhibition and increased D1 stimulation may resemble prefrontal hypoactivation. Attentional deficits caused by prefrontal disinhibition converge with recent findings that prefrontal disinhibition disrupts cognitive flexibility, another key aspect of prefrontal function (Gruber et al., 2010; Enomoto et al., 2011). Therefore, some prefrontal-dependent cognitive functions, including attention, require appropriately tuned prefrontal neuron activity, with both too much and too little activation causing deficits, as was originally suggested based on prefrontal single-unit recordings in monkeys performing a prefrontal-dependent working memory task (Goldman-Rakic, 1995; Rao et al., 2000). In contrast, response control is impaired by prefrontal hypoactivation (present study; Paine et al., 2011; Murphy et al., 2012) or lesions (Chudasama and Muir, 2001; Passetti et al., 2002; Dalley et al., 2004; Chudasama and Robbins, 2006; Pezze et al., 2009; Chudasama et al., 2012), whereas disinhibition does not change (present study; Paine et al., 2011) or, if ventral prefrontal cortex is affected, even improves response control (Murphy et al., 2012). Therefore, response control requires prefrontal neural activity, but not the appropriate tuning of such activity.

Other behavioral effects
Prefrontal muscimol dose dependently reduced, whereas the highest picrotoxin dose (300 ng) increased locomotor activity, consistent with previous reports of locomotor effects of prefrontal muscimol (Marquis et al., 2007; Paine et al., 2011) or GABA-A antagonists (Matsumoto et al., 2003; Enomoto et al., 2011) and with prefrontal disinhibition increasing midbrain dopamine neuron activity and striatal dopamine release (which stimulates locomotor activity; Karreman and Moghaddam, 1996; Matsumoto et al., 2003; Enomoto et al., 2011; Lodge, 2011). Importantly, the locomotor effects cannot explain the 5CSRT deficits. First, key 5CSRT performance measures were affected at muscimol and picrotoxin doses that did not affect locomotor activity. Second, prefrontal muscimol and picrotoxin had opposite locomotor effects, but both caused attentional deficits.

Neither prefrontal hypoactivation nor disinhibition substantially affected startle or PPI in our Lister hooded rats, whereas PPI in Sprague Dawley rats was nearly halved by prefrontal infusion of a comparatively low picrotoxin dose (10 ng/0.5 µl/side; Japha and Koch, 1999). Although strain (and species)-dependent PPI modulation poses problems for the translational use of PPI, and the functional and the clinical significance of PPI remains to be clarified (Swerdlow et al., 2008), our data dissociate the cognitive deficits caused by prefrontal hypoactivation and disinhibition from changes in basic startle and PPI measures.

In vivo electrophysiological findings
The inhibiting effects of muscimol and disinhibiting effects of picrotoxin on prefrontal neurons were clearly revealed by reduced and increased, respectively, within-burst firing rates. Such inhibition and disinhibition of prefrontal firing may interfere with cognitive processing by disrupting appropriate neural tuning, with inhibition reducing neuronal responses to relevant stimuli and disinhibition increasing responses to irrelevant stimuli.

The changes in the prevalence and temporal pattern of bursting that were induced by muscimol and picrotoxin may also have important cognitive/behavioral implications. First, task-appropriate neural information processing may require an appropriate balance of burst and nonburst single-spike firing patterns (Cooper, 2002; Larkum, 2013) and specific temporal patterns of burst firing (Izhikevich et al., 2003). Therefore, altered bursting may partly account for the attentional deficits caused by prefrontal muscimol and picrotoxin. Interestingly, 300 but not 150 ng picrotoxin changed the balance of burst and nonburst firing (increased percentage of spikes fired in bursts) and this corresponded to more pronounced attentional deficits (with accuracy decreased and omissions increased compared with only decreased omissions at 150 ng). Second, burst-like high-frequency (60 Hz) prefrontal stimulation increases, whereas low-frequency (10 Hz) stimulation decreases ventral striatal dopamine release (Jackson et al., 2001) and clinically efficient antipsychotics reduce prefrontal burst firing (Wang and Goldman-Rakic, 2004). This suggests that enhanced prefrontal bursting, as caused by reduced GABA function, may contribute to dopamine hyperfunction and be relevant to psychosis. Interestingly, picrotoxin increased locomotor activity only at 300, but not 150 ng, which may reflect the different effects on bursting: 300 ng enhanced prefrontal burst firing, but reduced spiking outside bursts, which should increase striatal dopamine. In contrast, 150 ng, although also enhancing bursting, did not reduce (or even slightly stimulated) low-frequency spiking outside bursts, which may decrease striatal dopamine transmission (Jackson et al., 2001) and thereby counteract locomotor hyperactivity. Altered ventral striatal dopamine may also disrupt attention, which depends on optimal accumbal dopamine receptor stimulation (Pezze et al., 2007).

Prefrontal picrotoxin markedly enhanced prefrontal LFP power averaged across the complete frequency range investigated (0.7–170 Hz), similar to recent findings after prefrontal bicuculline infusions (Lodge, 2011). Extrapolations to awake human EEG have to be made with caution, because LFP patterns in anesthetized rats differ substantially from awake human EEG, and the disinhibition-induced spike waves we recorded are largely restricted to slow-wave sleep or anesthesia (Steriade and Contreras, 1998). However, basic mechanisms of LFP/EEG enhancement under anesthesia have been suggested to be relevant during wakefulness (Haider and McCormick, 2009). Therefore, although the prefrontal LFP enhancement may require confirmation by awake recordings, this finding indicates that prefrontal disinhibition may contribute to enhanced frontal resting EEG power, a well documented biomarker of schizophrenia (Winterer et al., 2004; Venables et al., 2009; Uhlhaas and Singer, 2012) that Winterer et al. (2004) reported to predict patients’ deficits on prefrontal-dependent tasks, including the continuous performance test of sustained attention.
Conclusions

Attention requires a balanced level of prefrontal activity, with both prefrontal hypoactivation and disinhibition causing a deficit, whereas for response control it may be sufficient that prefrontal activity is maintained above a minimal level. Drugs restoring balanced prefrontal activity may ameliorate deficits in prefrontal-dependent attention, whereas drugs that simply boost prefrontal activation (to antagonize hypoactivation) or reduce prefrontal activity (to antagonize disinhibition) are probably less useful, because the treatment effect may “overshoot,” causing too much or too little prefrontal activation and thereby disrupting attention. Attention’s high susceptibility to disruption by aberrant prefrontal activity implies that dysfunction in areas with strong prefrontal connectivity, such as hippocampus, may induce attentional deficits (Bast, 2011) and we recently found evidence supporting this (McGarry et al., 2013). Prefrontal disinhibition caused additional schizophrenia-relevant effects, namely psychosis-related enhanced prefrontal bursting and locomotor hyperactivity, and enhanced prefrontal LFP power, which may be relevant to increased frontal background EEG in patients. This supports a key role of prefrontal disinhibition in causing schizophrenia-related neurobehavioral abnormalities (Enomoto et al., 2011; O’Donnell, 2011).

References

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