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ORIGINAL ARTICLE

Hippocampal Neural Disinhibition Causes Attentional and Memory Deficits

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Abstract

Subconvulsive hippocampal neural disinhibition, that is reduced GABAergic inhibition, has been implicated in neuropsychiatric disorders characterized by attentional and memory deficits, including schizophrenia and age-related cognitive decline. Considering that neural disinhibition may disrupt both intra-hippocampal processing and processing in hippocampal projection sites, we hypothesized that hippocampal disinhibition disrupts hippocampus-dependent memory performance and, based on strong hippocampo-prefrontal connectivity, also prefrontal-dependent attention. In support of this hypothesis, we report that acute hippocampal disinhibition by microinfusion of the GABA-A receptor antagonist picrotoxin in rats impaired hippocampus-dependent everyday-type rapid place learning performance on the watermaze delayed-matching-to-place test and prefrontal-dependent attentional performance on the 5-choice-serial-reaction-time test, which does not normally require the hippocampus. For comparison, we also examined psychosis-related sensorimotor effects, using startle/prepulse inhibition (PPI) and locomotor testing. Hippocampal picrotoxin moderately increased locomotion and slightly reduced startle reactivity, without affecting PPI. In vivo electrophysiological recordings in the vicinity of the infusion site showed that picrotoxin mainly enhanced burst firing of hippocampal neurons. In conclusion, hippocampal neural disinhibition disrupts hippocampus-dependent memory performance and also manifests through deficits in not normally hippocampus-dependent attentional performance. These behavioral deficits may reflect a disrupted control of burst firing, which may disrupt hippocampal processing and cause aberrant drive to hippocampal projection sites.

Key words: 5-choice-serial-reaction-time test, GABA inhibition, hippocampus, rat, watermaze delayed-matching-to-place test

Introduction

Attentional and memory deficits cause substantial functional disability and are a major treatment challenge in neuropsychiatric disorders, including schizophrenia and age-related cognitive decline (Millan et al. 2012). GABAergic inhibitory neurotransmission is important to shape brain activity, and subconvulsive cortico-hippocampal neural disinhibition, that is impaired GABAergic inhibition, has been implicated in neuropsychiatric disorders and associated cognitive deficits (Marin 2012). Here, we focus on hippocampal neural disinhibition, aiming to examine its causal contribution to attentional and memory deficits. Hippocampal neural disinhibition has emerged as a key pathophysiological feature of schizophrenia, based on consistent findings of metabolic overactivity at rest and altered post-mortem markers of γ-aminobutyric acid (GABA) function in the hippocampus of schizophrenia

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patients (Lisman et al. 2008; Heckers and Konradi 2015; Ruzicka et al. 2015). Hippocampal GABA dysfunction has also been implicated in other cognitive disorders, notably age-related cognitive decline (Huang and Mucke 2012; Stanley et al. 2012; Nava-Mesa et al. 2014), although the evidence is more preliminary than the evidence implicating GABA dysfunction in schizophrenia.

Neural disinhibition, by disrupting balanced neural activity within the disinhibited region, may impair local cognitive processing. This has recently been established for prefrontal GABA dysfunction (Gruber et al. 2010; Enomoto et al. 2011; Paine et al. 2011, 2015; Pehrson et al. 2013; Pezze et al. 2014; Tse et al. 2015), and there is also evidence linking hippocampal neural disinhibition and overactivity to impaired hippocampus-dependent memory performance (Koh et al. 2010; Murray et al. 2011; Andrews-Zwilling et al. 2012; Bakker et al. 2012 ; Caputi et al. 2012; Gilani et al. 2014; Lovett-Barron et al. 2014). Moreover, regional disinhibition, by causing aberrant drive of projections, may disrupt neural activity and cognitive processing in distal sites. Consistent with distal effects, rodent studies show that hippocampal disinhibition and overactivity cause dopamine system upregulation and associated behavioral effects (including locomotor hyperactivity) (Bast et al. 2001a; Bast and Feldon 2003; Lodge and Grace 2011; Gilani et al. 2014), which—given the strong link between dopamine and psychosis (Howes and Kapur 2009)—may explain the consistent correlation of hippocampal overactivity and psychosis in schizophrenia (Liddle et al. 1992; Schobel et al. 2009; Heckers and Konradi 2015). Considering local and distal effects, we hypothesized that hippocampal neural disinhibition impairs hippocampus-dependent memory and, based on strong hippocampo-prefrontal connectivity, also prefrontal-dependent cognitive function, including attention (Bast 2011). This hypothesis would explain recent imaging findings that intrinsic hippocampal overactivity correlates with memory and attentional deficits in schizophrenia (Tregellas et al. 2014).

To test if hippocampal neural disinhibition disrupts hippocampus-dependent memory and prefrontal-dependent attention, we combined hippocampal neural disinhibition by local microinfusion of the GABA-A antagonist picrotoxin (Bast et al. 2001a; Pezze et al. 2014) with translational tests of clinically relevant cognitive deficits in rats. Infusions targeted temporal (also referred to as ventral) to intermediate hippocampus, because this part of the hippocampus features strong hippocampo-prefrontal connectivity and corresponds to human anterior hippocampus, which has been implicated in schizophrenia (Bast 2011). To test for attentional deficits, we used the 5-choice-serial-reaction-time (5-CSRT) task, which has high validity to measure prefrontal-dependent sustained attention, as impaired in several cognitive disorders, including schizophrenia and age-related cognitive decline (Chudasama and Robbins 2006; Lustig et al. 2013; Romberg et al. 2013), and requires balanced prefrontal activity (Pezze et al. 2014). To test for memory deficits, we used the watermaze delayed-matching-to-place (DMP) task, which requires rats to learn rapidly (within one trial) new place information everyday and is highly dependent on hippocampal function (Steele and Morris 1999; Bast et al. 2009; Pezze and Bast 2012), including function of the temporal to intermediate hippocampus (presumably because these regions feature functional connectivity to frontal and subcortical sites necessary to translate hippocampal learning into performance; Bast et al. 2009; Bast 2011). The task resembles the everyday problem of remembering new places and routes, and similar human tests using virtual or real-space analogs of the watermaze revealed marked deficits in schizophrenia and age-related cognitive decline (Hort et al. 2007; Fajnerova et al. 2014). For comparison and to identify behaviorally effective doses without gross sensorimotor effects, we first examined effects on locomotor activity and startle prepulse inhibition (PPI), with locomotor hyperactivity and PPI disruption also being widely used psychosis-related indices (Bast and Feldon 2003; Swerdlow et al. 2008). To examine neural changes caused by hippocampal GABA dysfunction, we conducted in vivo electrophysiological recordings in the vicinity of the infusion site (Pezze et al. 2014). Of particular interest were changes in hippocampal burst firing, which has been proposed to be important for hippocampus-dependent memory function (Takahashi and Magee 2009; Xu et al. 2012) and for driving postsynaptic targets (Lisman 1997) and has recently been suggested to depend critically on local GABAergic inhibition, with opto- and pharmacogenetic silencing of GABAergic interneurons enhancing bursting (Lovett-Barron et al. 2012; Royer et al. 2012).

Materials and Methods

Animals

Adult male Lister hooded rats (Charles River) were used for all studies: 31 for the sensorimotor experiments (2–3 months at surgery), 11 for the 5-CSRT experiment (6–7 months at surgery), 14 for the watermaze experiments (2–3 months at surgery), and 15 for the electrophysiology (2–3 months at time of the acute experiment). Rats were housed in groups of 4 in 2 level “Double Decker” cages (462 mm × 403 mm × 404 mm; Tecniplast) under temperature- and humidity-controlled (21 ± 1.5 °C; 50 ± 8%) conditions and alternating 12 h light dark cycle (lights on at 0700 h). They had ad libitum access to water and food (Teklad Global 18% Protein Rodent Diet 2018, Harlan), except for the rats used in the 5-CSRT experiment, which were kept on a restricted diet to keep their weight at about 85% of their projected bodyweight during behavioral testing. All rats were habituated to handling by the experimenters before the start of any experimental procedures. All procedures were conducted in accordance with the requirements of the United Kingdom (UK) Animals (Scientific Procedures) Act 1986.

Cannula Implantation for Behavioral Studies

Using stereotactic procedures described in detail previously (Pezze et al. 2014), guide cannulae (stainless steel, 26-gauge, protruding 8.5 mm from a plastic pedestal; Plastics One, Bilaney) with stylets (stainless steel, 33 gauge; Plastics One, Bilaney) protruding 0.5 mm were implanted into the temporal to intermediate hippocampus, with the stylets aiming at (in mm): 5.2 posterior to bregma, ±4.8 lateral from midline, and 6.5 ventral from dura. These coordinates were adapted from our previous study in Wistar rats (Bast et al. 2001a) based on pilot surgeries. After surgery, rats were given at least 4 days of recovery before any testing. Throughout the recovery period, rats underwent daily health checks and were habituated to the manual restraint necessary for the drug microinfusions.

At the end of the experiments, brains were perfusion-fixated and processed histologically, as described previously (Pezze et al. 2014), to verify placements of the infusion cannulae and map them onto coronal sections adapted from a rat brain stereotaxic atlas (Paxinos and Watson 1998).

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Microinfusions for Behavioral Studies

Rats were manually restrained during the infusions. Stylets were replaced by infusion cannulae (protruding 0.5 mm from tip of guide cannulae, stainless steel, 33 gauge; Plastics One, Bilaney), which were connected via flexible polyethylene tubing to 5-μL SGE microsyringes mounted on a microinfusion pump (sp200IZ, World Precision Instruments). A volume of 0.5 μL/side of 0.9% sterile saline (as control) or of a solution of picrotoxin ($C_{30}H_{34}O_{13}$; Sigma-Aldrich) in saline (25–150 ng/0.5 μL/side, depending on experiment) was then infused bilaterally over 1 min. The picrotoxin solutions were prepared on the day of the experiments from frozen aliquots containing 150 ng/0.5 μL. Movement of an air bubble, trapped in the tubing, was used to monitor the infusion flow. Infusion cannulae were withdrawn and replaced by stylets 60 s after completion of the infusion to allow for absorption of the infusion bolus. Behavioral testing started immediately after infusions had been completed and stylets reinserted, except for sensorimotor testing, for which rats were infused in pairs, one after the other, resulting in half of the rats having a delay of about 3–5 min between the end of the infusion and the start of testing.

In the 5-CSRT and watermaze experiments, mock infusions were performed on all rats after pretraining to asymptotic performance levels and before the actual infusion experiments. Mock infusions served to habituate the rats to the infusion procedure and to verify that the infusion procedure itself does not interfere with task performance. Mock infusions were conducted in the same way as real infusions, except that the pump was not connected to the syringes.

The picrotoxin doses used in the present study were subconvulsive, as we aimed to investigate the neurocognitive sequels of neural disinhibition relevant to neuropsychiatric disorders, not epileptic seizures. As in our previous studies involving hippocampal picrotoxin infusions (Bast et al. 2001a), observations of the rats following infusions and between infusion days did not reveal infusion-induced motor convulsions or more subtle indicators of seizure development, such as facial twitches, tremor, movement arrest or wet-dog shakes, which may result from higher doses of GABA antagonists (Bragin et al. 2009). Furthermore, our electrophysiological studies did not indicate electrophysiological seizure signs (see below).

Startle, PPI and Locomotor Activity Testing

We began by examining the effects of hippocampal picrotoxin on 3 basic sensorimotor process, the acoustic startle response and PPI of the acoustic startle response and open-field locomotor activity, similar to our previous studies in Wistar rats (Bast et al. 2001a). First, these experiments allowed us to confirm suitable picrotoxin doses for use in the Lister hooded strain that were behaviorally effective without causing gross sensorimotor impairment. Second, disrupted PPI of the acoustic startle response and locomotor hyperactivity are widely used psychosis-related indices in rodent studies (Bast and Feldon 2003; Arguello and Gogos 2006). PPI of the acoustic startle response refers to the reduction of the startle response to an intense acoustic pulse by an immediately preceding weaker, nonstartling, prepulse. It may reflect sensorimotor gating processes, which are disrupted in schizophrenia and restored by antipsychotic medication (even though PPI disruption is not specific to schizophrenia and the relation of PPI deficits to symptoms is not clear, Swerdlow et al. 2008). Startle, PPI and locomotor tests were conducted as described in detail previously, using between-subjects designs (Pezze et al. 2014).

Nineteen rats, implanted with hippocampal cannulae, were used to test the effects of hippocampal picrotoxin on startle and PPI (test session lasted 23 min, including 5 min of acclimatization). On day 1, startle and PPI were measured without infusion. On day 2, startle and PPI were measured following infusions of saline (n = 9) or 150 ng/side picrotoxin (n = 10). On Day 3, rats were re-tested without infusion as on day 1.

Twenty-nine preimplanted rats were used for locomotor testing. The same rats used for the startle/PPI experiments (except for 2 rats which fell ill) were used, 1 week later, to compare the locomotor effects of hippocampal infusion of saline (n = 6) and of 100 ng (n = 5) or 150 ng picrotoxin (n = 6) (i.e., these rats received a total of 2 infusions, with some rats receiving the same infusion as during the startle/PPI experiment and others receiving a different infusion, so as to match the new infusion groups with respect to their infusion history). Consistent with previous experiments in Wistar rats (Bast et al. 2001a), both 100 and 150 ng/side of picrotoxin ncreased locomotor activity. Therefore, we also examined the effects of lower doses, using an additional 12 experimentally naïve rats, receiving either saline (n = 4), 50 ng (n = 4) or 75 ng (n = 4) picrotoxin. On day 1, baseline locomotor activity was tested for 1 h. On day 2, following 30 min of preinfusion testing, rats received infusions of saline (n = 10), 50 ng (n = 4), 75 ng (n = 4), 100 ng (n = 5) or 150 ng (n = 6) picrotoxin and an additional 60 min of postinfusion testing. On day 3, rats were re-tested without infusions as on day 1.

The purpose of locomotor and startle/PPI testing without infusion on days 1 and 3 was to verify that there were no baseline differences in dependent measures between groups before infusions, and that any group differences were reflecting temporary infusion-induced changes. None of the sensorimotor measures showed any group differences on days 1 and 3 (data not shown).

Data were analyzed using ANOVA with infusion group as between-subjects factor and 10-min bins (locomotor activity), test block (startle), or prepulse intensity (for PPI data) as within-subjects factor.

5-CSRT Experiment

The 5-CSRT test requires rats to sustain and divide attention across a row of 5 apertures to detect brief light flashes occurring in random order in one of the apertures and to respond to these flashes by nose-poking into the correct hole to receive food reward. Procedures were described in detail previously (Pezze et al. 2014).

Test boxes had 5 holes on one side and a food magazine on the opposite wall. Nose pokes into holes and magazine were detected using infrared beams. Test sessions started with delivery of one food pellet. Rats triggered a trial by nose-poking into the magazine. The trial started after a 5 s delay (intertrial interval; ITI), with a light appearing in one of the holes for a 0.5 s stimulus duration. If the rat nose poked into that hole within a 5 s limited hold period (correct response), a reward pellet was released into the magazine. Responses in one of the unlit 4 holes (incorrect response), failure to respond within the limited hold period (omission), and responses during the ITI (premature response) were punished by a 5-s time-out period with the house light turned off. Repeated responses in the same hole (correct or incorrect) were recorded as perseverative responses. A new trial started 5 s after the rat entered the food magazine,

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either to collect the reward or after the 5-s time-out. Test sessions consisted of 100 trials or lasted 30 min, whichever was shorter. Each rat had only one test session per day.

The main measures of attention were: % accuracy ([correct responses/(correct responses + incorrect responses)] × 100%), reflecting errors of commission due to faulty stimulus detection, and % omissions ([omissions/(correct responses + incorrect responses + omissions)] × 100%), which may reflect failure to detect the stimulus. As measures of response control, we analyzed % premature responses ([premature responses/(correct responses + incorrect responses + omissions + premature responses)] × 100%) and % perseverative responses ([perseverative responses/(correct responses + incorrect responses + omissions + premature responses)] × 100%), reflecting failure to withhold prepotent, but inappropriate, responses. Additional measures, to control for nonspecific motor and/or motivational changes, were: 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).

Rats were pretrained before and retrained after surgery to perform at stable and high performance levels (at least 80 correct trials, with 80% accuracy and 20% omissions) for at least 5 consecutive days. Rats then underwent 4 days of testing combined with mock infusions, with half of the rats receiving mock infusions on day 2 and the other half on day 4. Mock infusions did not affect task performance (data not shown). Eleven rats were used to test the effects of hippocampal picrotoxin infusions in within-subjects studies, with testing order of 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). We tested 2 dose ranges, each including saline plus 2 picrotoxin doses. The lower dose range included saline, 25 and 75 ng picrotoxin per side (chosen because they were at the low end of doses causing moderate locomotor hyperactivity in our sensorimotor experiments). Testing this dose range in the first few rats indicated no substantial effect on 5-CSRT performance. Therefore, we included an additional higher dose range, including saline, 75 and 150 ng per side (75 ng dose was included in both ranges as an internal control for reproducibility of drug effects). Of the 11 rats, 9 completed both dose ranges (receiving a total of 6 infusions, including 2 saline infusions), with testing at the 2 dose ranges separated by a minimum of 4 days of testing without infusion (to re-establish a stable baseline) and testing order counterbalanced across rats. In addition, one rat each completed only the higher or lower dose range (because of illness). Thus, each dose range was tested in 10 rats. Data were analyzed separately for both dose ranges by ANOVA, using drug dose as within-subjects factor, followed by post-hoc comparisons using Fisher's LSD (least significant difference) test (which provides good power and, if preceded by ANOVA and if only 3 means are compared as in the present study, ensures that the familywise type-1 error rate is equal or lower than the rate for the individual comparison; Levin et al. 1994).

Watermaze DMP Experiment

The watermaze DMP task requires rats to learn rapidly, within one trial, the daily changing place of a hidden platform in order to escape efficiently from a circular pool of water (Steele and Morris 1999). The task, especially in our new modification measuring search preference on probe trials to assess one-trial place memory, is highly sensitive to disruption of hippocampal function (Bast et al. 2009; Pezze and Bast 2012). Procedures were adapted from our previous study examining the effects of hippocampal drug microinfusions on the watermaze DMP test (Pezze and Bast 2012).

Apparatus

The watermaze consisted of a 2-m diameter white fiberglass pool, which was 60 cm high and filled with water up to 20 cm below the pool edge. It was positioned in a well-lit (200 lux) room, which contained a variety of extra-maze visual cues visible from the water surface, so as to aid spatial orientation, including high-contrast wall posters and 3-dimensional visual cues (cupboards, a traffic cone, lampshades and boxes hanging from the wall) arranged at various distances from the pool. Four points, equally spaced along the circumference of the pool [North (N), East (E), South (S) and West (W); arbitrarily defined], served as start positions. The water was made opaque with latex (200 mL; Febflor Latex Liquid, Everbuild Building Products Ltd). During testing, water temperature was maintained at 25 ± 1 °C. The rats' only way to escape from the water was via a 12-cm diameter escape platform, which was hidden from the rats' sight 1–2 cm below the water surface.

We used a so-called "Atlantis platform" (Spooner et al. 1994), which can be withheld at >20 cm below the water surface, inaccessible for the rats, by a computer-controlled electromagnet for a predetermined time, before rising to its normal position (Med Associates, Inc.). This allowed us to run rewarded probe trials during which the rats' search preference for the zone containing the platform location was first monitored for 60 s before the platform was made available to reinforce spatially focused searching.

To record the rats' swimming behavior, a digital camera was mounted above the watermaze, transmitting images to a PC in the adjacent room. The PC ran a program for video capture of the trials (Pinnacle Studio 12, Pinnacale Systems, Corel Corporation) and the Ethovision tracking software (Version XT 7, Noldus Technology) that digitizes the rats' paths and can compute various behavioral measures, including latencies and path lengths to reach the platform location, and times in different areas of the pool.

General Testing Procedure

Rats received 4 trials a day. The platform was hidden in a new place on trial 1 of each day, remaining in this place for trials 2–4, on which rats could use rapidly encoded place memory to reach the escape platform efficiently. Each of the 4 start positions around the pool (N, E, S, W) was used daily in an arbitrary, predetermined, sequence to discourage egocentric strategies. Analysis focused on trial 2 of each day, when performance relies on place memory encoded within trial 1, whereas trials 3 and 4 mainly served to reinforce the task‘s win-stay rule. Trial 2 was occasionally run as rewarded probe trial. During such probe trials, the Atlantis platform was withheld at the bottom of the pool for 60 s, to enable the measurement of search preference for the "correct" zone containing the platform location (see below, Performance measures), and was then released automatically (i.e., independent of the rat's behavior) in order to allow the rat to find and climb onto the platform and to reinforce the win-stay rule of the task, that is that the rat had to return to the correct location to escape from the pool. Each trial began with the rat being gently lowered into the water facing the pool wall at one of the 4 start positions. Ethovision tracking was triggered by remote control as soon as the rat was

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released into the water. If rats failed to reach the platform within 120 s, they were guided to the platform by the experimenter (i.e., 120 s was the maximum latency to reach the platform location). Rats were left on the platform for 30 s, before being removed from the pool and placed onto a towel in an opaque plastic box close to the entrance of the watermaze room until the start of the next trial. The center of the escape platform was either located on an inner (0.8 m) or outer (1.4 m) ring concentric with the pool. Rats were tested with a novel location each day: as each experiment involved 20 days of training or testing (8 days of pretraining plus 2 series of 6 days during which the effects of hippocampal drug infusions were tested, see below, Experimental design), 20 different locations were used (see Fig. 1C in Pezze and Bast, 2012). The ITI was usually 10–30 s (i.e., as short as possible for convenience), but on selected test days the ITI, or retention delay, between trials 1 and 2 was increased to 20 min (see below, Experimental design), similar to previous studies showing disruptive effects of hippocampal pharmacological manipulations (Steele and Morris 1999; Pezze and Bast 2012).

Performance Measures

Search preference in the vicinity of the platform location during probe trials was used as the main measure of rapid, 1-trial, place memory performance, based on evidence from our previous studies that search preference is more sensitive than latency and path length measures (including savings, i.e., trial 1 values—trial 2 values) to manipulations of the hippocampus (Bast et al. 2009; Pezze and Bast 2012) and to behavioral manipulations thought to affect hippocampus-dependent memory (da Silva et al. 2014). The main reason for the lower sensitivity of path lengths measures is that they have a much higher variability (with trial one path lengths in particular being highly chance dependent) than search preference (compare da Silva et al., 2014). Therefore, since introduction of probe trials as part of the DMP procedure (Bast et al., 2009), we have been using search preference as the main performance measure in our watermaze DMP experiments (as has long been the case for the standard watermaze paradigm). To quantify search preference, eight 40-cm diameter “virtual” zones were defined on the inner and outer ring of the pool, so that one zone, the “correct” zone, was concentric with the platform location, and all 8 zones were nonoverlapping, evenly spaced, and symmetrically arranged. The time spent in each of these 8 zones during the 60-s probe trial was determined automatically using the Ethovision software. From these measures, the “% time spent in the correct zone” was calculated as: [time in “correct zone” (s)/time in all 8 zones (s)] x 100%. By chance, this value should be 100%/8 = 12.5%, whereas higher values indicate a search preference for the correct zone. In addition, latencies and path lengths to reach the platform perimeter were recorded for all trials, with steep reduction from trial 1 to trial 2 indicating 1-trial place memory. Path lengths have the advantage over latencies that they measure the efficiency in reaching the platform independent of potential drug-induced swim speed changes. Therefore, on infusion days, analysis focused on path lengths.

Experimental Design to Test the Effects of Hippocampal Picrotoxin Infusions

Fourteen rats, preimplanted with hippocampal guide cannulae, were pretrained on the task for 8 days, using a novel location each day, before testing infusion effects. Rats were divided in 2 batches to be trained with 1 of 2 different sequences of daily platform locations, so that on each day 2 different platform locations were used for pretraining; the purpose of this was to reduce the risk that a day’s performance measures are biased by the properties of specific platform locations. The ITI between trial 1 and trial 2 was 10–30 s (i.e., as short as possible for convenience, because it reduced the time required for training) for the first 4 days of pretraining and 20 min for the remaining days. The ITIs for trials 2–4 were always 10–30 s for convenience. On pretraining days 6 and 8, trial 2 was run as probe. In addition, rats received a mock infusion immediately before trial 1 on either day 6 or 8. Half of the rats received the mock infusion on day 6, the other half on day 8, with start positions and platform locations counterbalanced between the mock-infusion and no-mock-infusion condition. As in our previous studies (Bast et al. 2009; Jackson et al. 2011; Pezze and Bast 2012; da Silva et al. 2014), rats showed asymptotic task performance at the end of pretraining, characterized by marked latency reduction from trial 1 to trial 2 and strong search preference on the probe trials, without any effect of mock infusions (data not shown).

Following pretraining, the effects of bilateral saline or picrotoxin (75 or 150 ng/side) infusions were then compared in a within-subjects design. Doses were chosen based on the 5-CSRT experiments, where 150 ng caused attentional deficits, and 75 ng was without effect. Each rat received 3 doses (including saline) across 3 infusion days, with each infusion day preceded by a testing day without infusion. This series of 3 infusions was repeated once more (resulting in a total of 6 infusions, including 2 saline infusions). The values from the 2 series were averaged to obtain one single value for each infusion condition (for one rat, values for all infusion conditions could only be collected in one series, and, therefore, for this rat, only the values from this one series were considered). On infusion days, rats received hippocampal infusions immediately before trial 1, and trial 2 was run 20 min after trial 1 as a probe to measure search preference. In both infusion series, testing order, as well as start positions and platform locations, was counterbalanced across the infusion conditions. The DMP task was run as during pretraining, with a new platform location, different from the pretraining locations, used everyday. As during pretraining, rats continued to be trained with 1 of 2 different sequences of daily platform locations, so that on each day 2 different platform locations were used.

Data Analysis

One average value for each performance measure in each infusion condition was calculated from the 2 series of infusions for each rat, before path length and search preference measures were subjected to ANOVA with infusion and trials (only for path lengths) as within-subject factors. Main effects were examined further using Fisher’s LSD test. To test for significant one-trial place memory, in each infusion condition, search preference was compared with chance (12.5%) and trial 1 and trial 2 path lengths were compared, using one-sample or paired sample *t*-tests (2-tailed), respectively.

In Vivo Electrophysiology to Measure the Effects of Hippocampal Microinfusions in the Vicinity of the Infusion Site

Multiunit and local field potential (LFP) activity in the vicinity of the infusion site was recorded under isofluorane anesthesia using a custom-made assembly of a 33-gauge stainless-steel

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infusion cannula and an 8-electrode (microwire) recording array. Methods were as described in detail in our previous study using such an infusion-recording array in the medial prefrontal cortex (Pezze et al. 2014), except that recording site and aspects of the data analysis were adapted for the hippocampus.

The infusion-recording assembly (see Fig. 5A) consisted of a 33-gauge stainless-steel infusion cannula attached to an 8-channel microwire array (eight 50-μm Teflon-coated stainless-steel wires, with an impedance of about 100 kΩ measured at 1 kHz and arranged in one row of about 2 mm) with a stainless-steel groundwire (NB Labs). The cannula tip touched the electrodes and was positioned about 0.5 mm above the tips of the central electrodes. The end of the cannula was connected to a 1-μL syringe via flexible tubing. Infusion cannula and tubing were filled with picrotoxin solution or saline before the infusion-recording assembly was inserted into the brain. A small air bubble was trapped where the tubing was connected to the syringe, with movement of the bubble serving to verify 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" separating the infusion solution from the brain's extracellular space. The assembly was stereotactically implanted into the right hippocampus, such that the electrode array was arranged perpendicular to the brain midline and anterior to the infusion cannula, with the cannula tip aimed at the same coordinates as in the behavioral experiments (5.2 mm posterior to bregma, 4.8 mm lateral from midline, and 6.5 mm ventral from dura).

To record extracellular measures of neural activity, the electrode array was connected via a unity-gain multichannel headstage to a multichannel preamplifier (Plexon, Inc.), which amplified (×1000) the analog signal and band-pass filtered it into multiunit spikes (250 Hz to 8 kHz) and LFP signals (0.7 Hz to 170 Hz). The analog signals were fed to a Multichannel Acquisition Processor (MAP) system (Plexon, Inc.). Multiunit and LFP data were viewed online and recorded with Real-Time Acquisition System Programs for Unit Timing in Neuroscience (RASPUTIN) software (Plexon, Inc.).

After positioning the assembly and stabilization (at least 30 min), LFP data were recorded continuously and multiunit spikes were recorded when a predefined amplitude threshold of −300 μV was crossed. Data were recorded for a 30 min baseline and a 60 min period following hippocampal infusion of 0.5 μL of saline (n = 7) or 150 ng picrotoxin in 0.5 μL saline (n = 8). For infusions, the piston of the 1-μL syringe was moved manually at a slow speed (ca. 0.5 μL/min as in the behavioral studies) to remove the 0.25-μL air plug from the injector tip and inject 0.5 μL of saline (n = 7) or of 150 ng picrotoxin in 0.5 μL saline (n = 8) into the hippocampus.

Following completion of the recordings, the rat was killed by increasing the isofluorane level. The positions of the most lateral and most medial electrodes were marked by passing cathodic current (0.2 mA, 10 s) to deposit ferric ions, which could be revealed by the Prussian Blue Reaction following fixation of the brain in a 4% paraformaldehyde solution containing 4% potassium ferrocyanide. Locations of the marked electrode tips were mapped onto coronal sections of the rat brain stereotaxic atlas by Paxinos and Watson (1998).

From the multiunit data, firing rate and burst parameters (number of bursts, % of spikes fired as bursts, mean firing rate within bursts, mean burst duration, interburst interval) were calculated in 5-min bins for each correctly placed electrode. Hippocampal bursts were defined as more than 2 spikes with an interspike interval shorter than 6 ms (Royer et al. 2012). From the LFP recordings, we calculated the area under the curve of the power spectral density function (AUC of PSD) from 0.7 to 170 Hz as a measure of overall LFP power for every 5-min block (Pezze et al. 2014). For normalization to baseline, values from individual channels were divided by the average values obtained from the same channel during the six 5-min baseline blocks. Normalized values were averaged across all channels per individual rat, and these average values were used to calculate means for the different infusion groups. Differences between infusion groups were examined, using ANOVA with infusion as between-subjects factor and 5-min block as within-subjects factor.

Results

Infusion Cannula Placements in Behavioral Studies

Infusion cannula tips were primarily placed within the temporal to intermediate hippocampus, within an area corresponding to 4.8–6.3 mm (5.6–6.3 mm in the Watermaze experiments) posterior to bregma in Paxinos and Watson (1998) (Fig. 1). In the locomotor and PPI experiments, the infusion sites tended to be slightly deeper than in the other 2 experiments, with several placements encroaching on the entorhinal cortex. Therefore, the locomotor and PPI effects in the present study may partly reflect the impact of picrotoxin within the entorhinal cortex. The entorhinal cortex features strong prefrontal and subcortical projections similar to temporal to intermediate hippocampus and previous studies indicate that stimulation or disinhibition of these sites has a similar impact on prefrontal cortex and subcortical sites (Lopes da Silva et al. 1990; Jay and Witter 1991; Gigg et al. 1994; Groenewegen et al. 1999; Mitchell et al. 2000; Floresco et al. 2001). Visible brain damage was restricted to the area immediately surrounding the guide cannula and the injection site (0.5 mm below the end of the guide).

Hippocampal Disinhibition Reduces Startle, Without Affecting PPI, and Increases Locomotor Activity

Hippocampal picrotoxin (150 ng/side) reduced startle reactivity on pulse-alone trials, especially during the first test block, before startle habituation led to similarly low startle amplitude in saline and picrotoxin groups (main effect of infusion: $F_{1,17}$ = 6.60, P = 0.020; interaction infusion × test block: $F_{2,34}$ = 6.85, P = 0.003) (Fig. 2A, left). Infusions did not affect PPI at any prepulse intensity (main effect of infusion: $F_{1,17}$ = 1.21, P = 0.29; interaction infusion × prepulse intensity: $F_{3,51}$ < 1) (Fig. 2A, right).

While all groups showed similar locomotor activity in the 30 min preceding infusions (main effect or interaction involving the factor infusion: both F < 1), picrotoxin (50, 75, 100 or 150 ng/side) infusions increased locomotor activity compared with saline, especially during the first 30–40 min of the 60-min post-infusion testing (main effect of infusion: $F_{4,24}$ = 6.11, P = 0.002; interaction infusion × 10-min block: $F_{20,120}$ = 2.86, P = 0.005) (Fig. 2B).

5-CSRT Experiment: Hippocampal Neural Disinhibition Causes Attentional Deficits

Hippocampal picrotoxin selectively impaired attention on the 5-CSRT test at the highest dose (150 ng), as indicated by reduced accuracy (Fig. 3), without significant effects on other performance measures or at other doses (Table 1). Testing at the higher dose range (saline, 75 and 150 ng) revealed a dose-dependent reduction of % accuracy ($F_{2,18}$ = 6.57, P = 0.007), with

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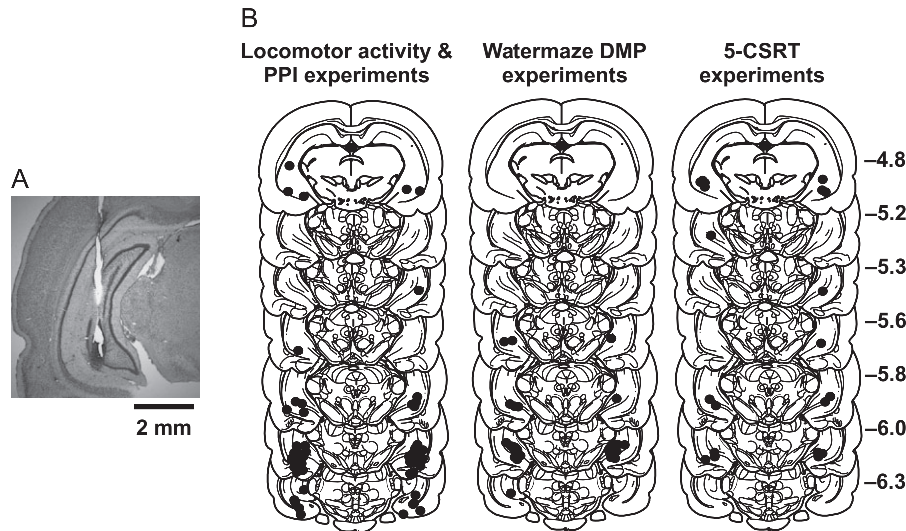

Figure 1. Infusion cannula placements in the behavioral studies. (*A*) Cresyl violet-stained section showing an exemplar infusion site in the temporal hippocampus. The section was taken from a rat that participated in the watermaze experiment. (*B*) Approximate locations of infusion cannula tips (dots) in the temporal to intermediate hippocampus, depicted separately for the different experiments. Locations are shown on coronal plates adapted from Paxinos and Watson (1998), with numbers on the right indicating distance from bregma in millimeters.

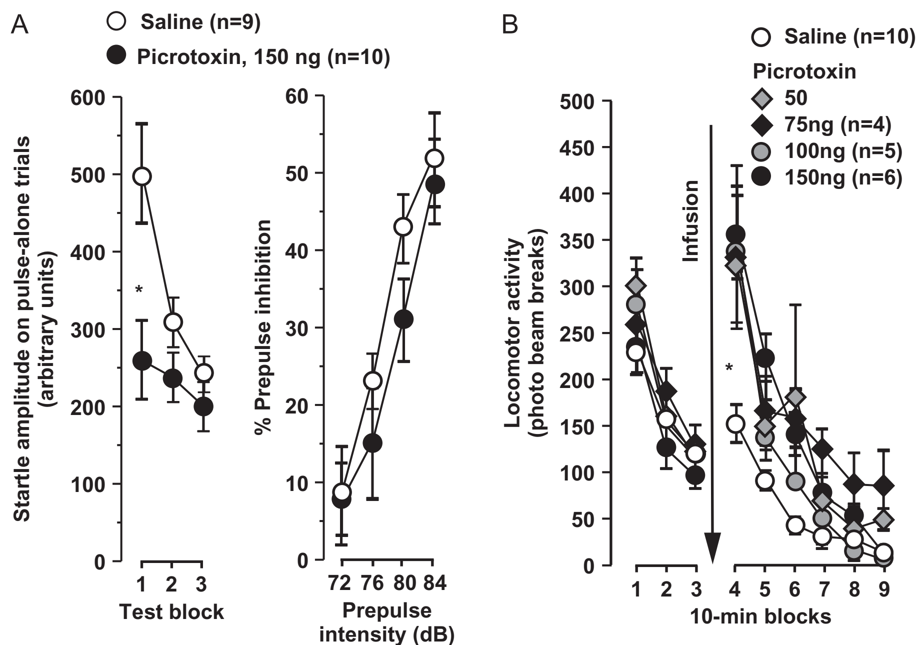

Figure 2. Sensorimotor effects of hippocampal neural disinhibition. (*A*) Hippocampal picrotoxin reduces startle reactivity, but does not affect PPI: Mean startle magnitude on pulse-alone trials (mean ± SEM) for the 3 test blocks (block 1, 10 consecutive pulse-alone trials; block 2, 10 pulse-alone trials interspersed with prepulse + pulse trials; block 3, 5 consecutive pulse-alone trials) and mean %PPI at the different prepulse intensities following infusion of saline or 150 ng of picrotoxin. Asterisk indicates significant interaction infusion × test block. (*B*) Hippocampal picrotoxin increases locomotor activity: Locomotor activity measured as photo beam breaks (mean ± SEM) did not differ between groups during the 30 min preceding infusion; hippocampal picrotoxin infusions (50, 75, 150 ng per side) increased activity as compared with saline infusion. Asterisk indicates significant interaction infusion × 10-min block.

150 ng reducing accuracy as compared with saline (P = 0.003) and 75 ng (P = 0.012), which did not differ from saline (P = 0.54).

Watermaze DMP Task: Hippocampal Disinhibition Impairs 1-Trial Place Memory Performance

Hippocampal picrotoxin impaired performance based on 1-trial place memory (Fig. 4). Search preference during probe trials was dose-dependently reduced ($F_{2,26}$ = 6.3, P = 0.006), with search preference at 150 ng significantly reduced as compared with saline (P = 0.002) and tending to be lower than at 75 ng (P = 0.05), which did not significantly differ from the saline condition (P = 0.15) (Fig. 4A). Search preference at 150 ng did not differ from chance (12.5%) (t_{13} < 1), whereas rats performed above chance at 75 ng (t_{13} = 2.2, P = 0.04) and with saline (t_{13} = 3.5, P = 0.005).

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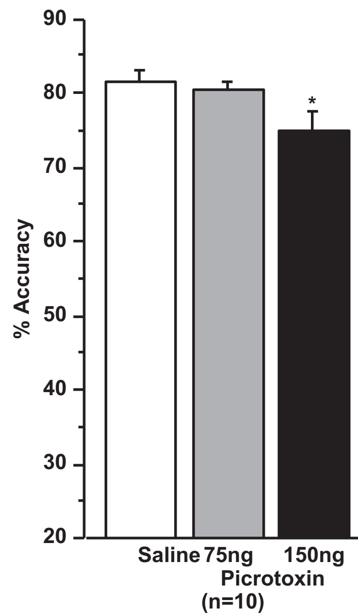

Figure 3. Hippocampal neural disinhibition causes attentional deficits on the 5-CSRT test. Attention, measured as % accuracy [(correct responses/(correct responses + incorrect responses)) × 100%, mean + SEM], was dose-dependently reduced by hippocampal picrotoxin infusion. Asterisk indicates significant difference as compared with saline condition.

Table 1. Performance measures (mean ± SEM) in the 5-CSRT experiments

Experiment	% Accuracy	% Omissions	% Premature responses	% Perseverative responses	Number of trials	Correct latency (s)	Collect latency (s)
Picrotoxin, 25 and 75 ng/side							
Saline	79.4 ± 1.9	11.3 ± 2.1	15.1 ± 3.8	9.8 ± 3.9	92.5 ± 3.8	0.58 ± 0.03	1.31 ± 0.12
25 ng	81.2 ± 2.2	10.2 ± 2.3	11.2 ± 1.8	11.8 ± 3.7	94.3 ± 3.9	0.57 ± 0.03	1.32 ± 0.11
75 ng	76.8 ± 3.1	12.2 ± 2.0	11.9 ± 1.7	8.4 ± 2.2	91.7 ± 4.0	0.61 ± 0.02	1.28 ± 0.10
Main effect of drug infusion	$F_{2,18} = 1.45$, $P = 0.26$	$F_{2,18} < 1$	$F_{2,18} < 1$	$F_{2,18} = 1.53$, $P = 0.24$	$F_{2,18} < 1$	$F_{2,18} < 1$	$F_{2,18} < 1$
Picrotoxin, 75 and 150 ng/side							
Saline	81.8 ± 1.1	14.1 ± 3.7	12.0 ± 1.8	7.0 ± 1.9	96.7 ± 1.9	0.60 ± 0.03	1.55 ± 0.17
75 ng	80.5 ± 1.2	11.8 ± 1.9	14.0 ± 2.1	8.5 ± 1.9	97.9 ± 1.7	0.59 ± 0.02	1.35 ± 0.13
150 ng	74.9 ± 2.8	14.4 ± 1.7	13.0 ± 3.8	6.4 ± 2.1	96.1 ± 2.5	0.64 ± 0.05	1.50 ± 0.18
Main effect of drug infusion	$F_{2,18} = 6.57$, $P = 0.007$	$F_{2,18} < 1$	$F_{2,18} < 1$	$F_{2,18} = 2.78$, $P = 0.09$	$F_{2,18} < 1$	$F_{2,18} < 1$	$F_{2,18} = 1.67$, $P = 0.22$

Path-length data also supported that hippocampal picrotoxin impaired performance based on rapid place learning (Fig. 4B). Path length analysis revealed an infusion effect ($F_{2,26} = 5.1$, $P = 0.01$), reflecting higher path lengths at the high picrotoxin dose, compared with saline (Saline vs. 150 ng, $P = 0.004$; Saline vs. 75 ng, $P = 0.12$; 75 ng vs. 150 ng, $P = 0.13$), and a trial effect ($F_{3,39} = 23.2$, $P < 0.0001$), reflecting path length reduction across trials. Although the dose × trial interaction was not significant ($F_{6,78} = 1.3$, $P = 0.29$), the increase of path lengths by picrotoxin, as compared with saline, appeared mainly driven by a less pronounced path-length reduction between trial 1 and trial 2, indicating impaired 1-trial place memory. Consistent with this, path lengths significantly decreased from trial 1 to trial 2 in the saline condition ($t_{13} = 4.36$, $P = 0.001$), but not at 75 ng ($t_{13} = 1.8$, $P = 0.09$) or 150 ng picrotoxin ($t_{13} < 1$). Picrotoxin infusions also numerically reduced path lengths savings from trial 1 to trial 2 (trial 1 path length—trial 2 path length, mean + SEM: Saline, 869.1 + 199.5 cm; 75 ng picrotoxin, 487.7 + 299.0 cm; 150 ng picrotoxin, 148.9 + 356.2 cm), although this effect was not significant ($F_{2,13} = 1.7$, $P = 0.20$). The pattern of infusion effects on latency measures was virtually identical to the one reported for the path length measures (data not shown). Thus, overall path length and latency measures support impairments in rapid place learning performance due to hippocampal picrotoxin infusion, although the relevant infusion effects on these measures failed to reach statistical significance. This is consistent with our previous studies showing that path length measures are less sensitive than the search preference measure to hippocampal manipulations (Bast et al. 2009; Pezze and Bast 2012) and to behavioral manipulations thought to affect hippocampus-dependent memory (da Silva et al. 2014).

To further characterize the nature of the impairment caused by hippocampal picrotoxin on the watermaze DMP test, we performed additional analyses of rats behavior during trial 1. First, during trial 1, rats spent a similar proportion of the trial

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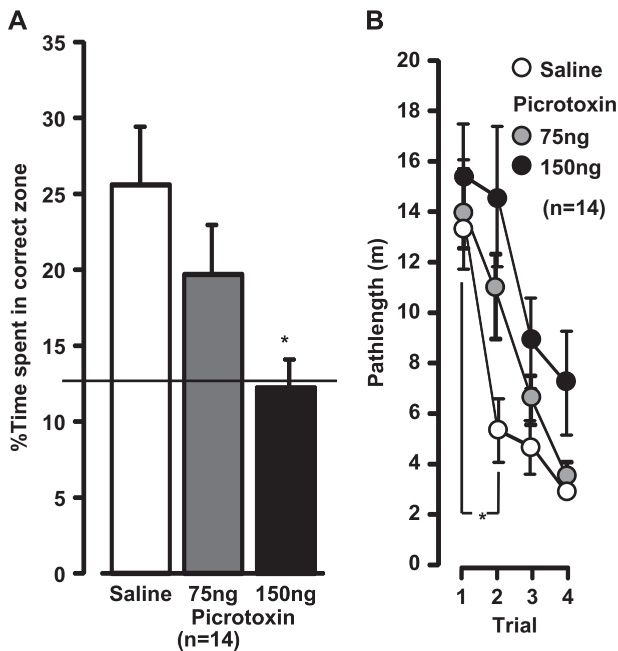

Figure 4. Hippocampal disinhibition impairs 1-trial place memory performance on the watermaze DMP test. (*A*) Percentage of time spent in the correct zone (mean + SEM) during probe trials was dose-dependently reduced by hippocampal picrotoxin infusion. The asterisk indicates a significant difference as compared with the saline condition. The horizontal line represents chance performance. (*B*) Path lengths (mean ± SEM) to reach the platform location were increased across all 4 trials following infusion of picrotoxin, with the most pronounced difference on trial 2. Rats only showed a significant path length reduction from trial 1 to trial 2 (indicated by asterisk) following saline infusion, but not following picrotoxin infusions.

duration (i.e., the time until rats reached the platform) in all 8 zones, regardless of the infusion condition [(time in all 8 zones/trial duration) × 100%, mean + SEM: Saline, 42.7 + 1.5%; 75 ng picrotoxin, 43.3 + 1.9%; 150 ng picrotoxin, 38.5 + 3.2%; $F_{2,26} = 1.2$, $P = 0.31$]. This pattern of results suggests that hippocampal neural disinhibition does not impair normal search behavior, consistent with informal observations of the rats' behavior during the experiment. Second, we analyzed which proportion of trial-1 time spent in these 8 zones was spent in the zone containing the platform location on the previous day (previous day's zone). Intact rats typically spend more time in the previous day's zone than expected based on chance (12.5%), indicating place memory for the previous day's correct location acquired during the 4 trials completed on the previous day (Steele and Morris, 1999). If picrotoxin-infused rats spent more time in the previous day's zone during trial 1, this could be taken to reflect perseverative behavior, which might interfere with learning of the new location. If they spent less time, this could reflect impaired retrieval/expression of place memory acquired during the 4 trials of the previous day (Steele and Morris, 1999). However, there was no significant effect of infusion on the percentage of time spent in the previous day's zone during trial 1 [(time in previous day's zone/total time in all 8 zones) × 100%, mean + SEM: Saline, 25.3 + 3.0%; 75 ng picrotoxin, 24.9 + 3.0%; 150 ng picrotoxin, 17.7 + 2.7%; $F_{2,26} = 2.3$, $P = 0.12$). These data show that hippocampal picrotoxin did not cause perseverative behavior on the watermaze test, consistent with the 5-CSRT data not revealing any perseverative tendencies (see Table 1). Hippocampal picrotoxin numerically reduced the time spent in the previous day's zone, mainly at the higher dose. Although ANOVA did not reveal a significant effect of infusion (see above), search preference in the previous day's zone was significantly above chance (12.5%) following hippocampal infusion of saline and 75 ng picrotoxin (both $t_{13} > 4.2$, $P < 0.001$), indicating memory for the previous day's location, but only tended to be higher than chance following infusion of 150 ng ($t_{13} = 1.9$, $P = 0.08$). Overall, these data support that hippocampal neural disinhibition does not markedly affect search behavior during trial 1, although the data points to a slight (nonsignificant) impairment in retrieval/expression of the place memory acquired during the previous day's 4 trials.

In Vivo Electrophysiology: Picrotoxin Enhances Hippocampal Neuron Firing and Bursting

The most medial and/or the most lateral electrodes were located outside of the hippocampus (typically 1–3 electrodes per rat). The data from these electrodes, which were placed just outside the medial or lateral boundaries of the hippocampus, were analyzed separately, providing a control measure for drug spread out of the hippocampus. All other electrodes were placed within the temporal to intermediate hippocampus (Fig. 5A).

At electrodes placed within the hippocampus, picrotoxin (150 ng) increased the overall firing rate and markedly enhanced the occurrence of bursts and the proportion of spikes fired as part of bursts, alongside a slight increase in burst duration, but without affecting within-burst firing rate (Fig. 5B). Mean preinfusion baseline values of the electrophysiological parameters analyzed did not differ between infusion groups (Table 2). Overall firing rates were increased by picrotoxin, as compared with saline, starting immediately after infusion, with firing rates in both groups converging again about 30 min after infusion because values in the picrotoxin group peaked between 10 and 25 min, while values in the saline group showed an upward drift (possibly reflecting nonspecific infusion effects or a baseline drift) between 15 and 25 min following infusion (interaction infusion group × 5 min block: $F_{17,221} = 1.85$, $P = 0.02$).

The most pronounced effects of picrotoxin on hippocampal neuron firing were increases in the occurrence of bursts and in the proportion of spikes fired as part of bursts. Picrotoxin markedly increased bursts per minute, starting within 5 min and peaking around 25–30 min after infusion before values converged with the saline group again (interaction drug x 5 min block: $F_{17,221} = 2.05$, $P = 0.01$). The percentage of spikes fired in bursts was also markedly increased, following a similar time course as bursts per minute (interaction infusion group × 5-min block: $F_{17,221} = 2.96$, $P < 0.001$). Moreover, picrotoxin increased burst duration, starting immediately after infusion and peaking 20–25 min later before values slowly returned to baseline (interaction infusion group × 5-min block: $F_{17,221} = 3.42$, $P < 0.001$). Consistent with the increased burst duration, picrotoxin decreased interburst intervals (interaction infusion group × block: $F_{17,221} = 1.88$, $P = 0.02$). Within-burst firing rate was not affected by infusions (main effect or interaction involving infusion group: $F < 1.4$, $P > 0.4$).

Consistent with the absence of behavioral seizure signs, LFP recordings did not show characteristics of hippocampal seizures that may result from convulsive doses of GABA-A antagonists, namely a fast sequence of so-called LFP "spikes" (sharp negative LFP deflection) at a rate of 5–20 per second superimposed on spike-wave discharges (a high-amplitude sharp negative LFP deflection followed by a positive LFP wave) (Bragin

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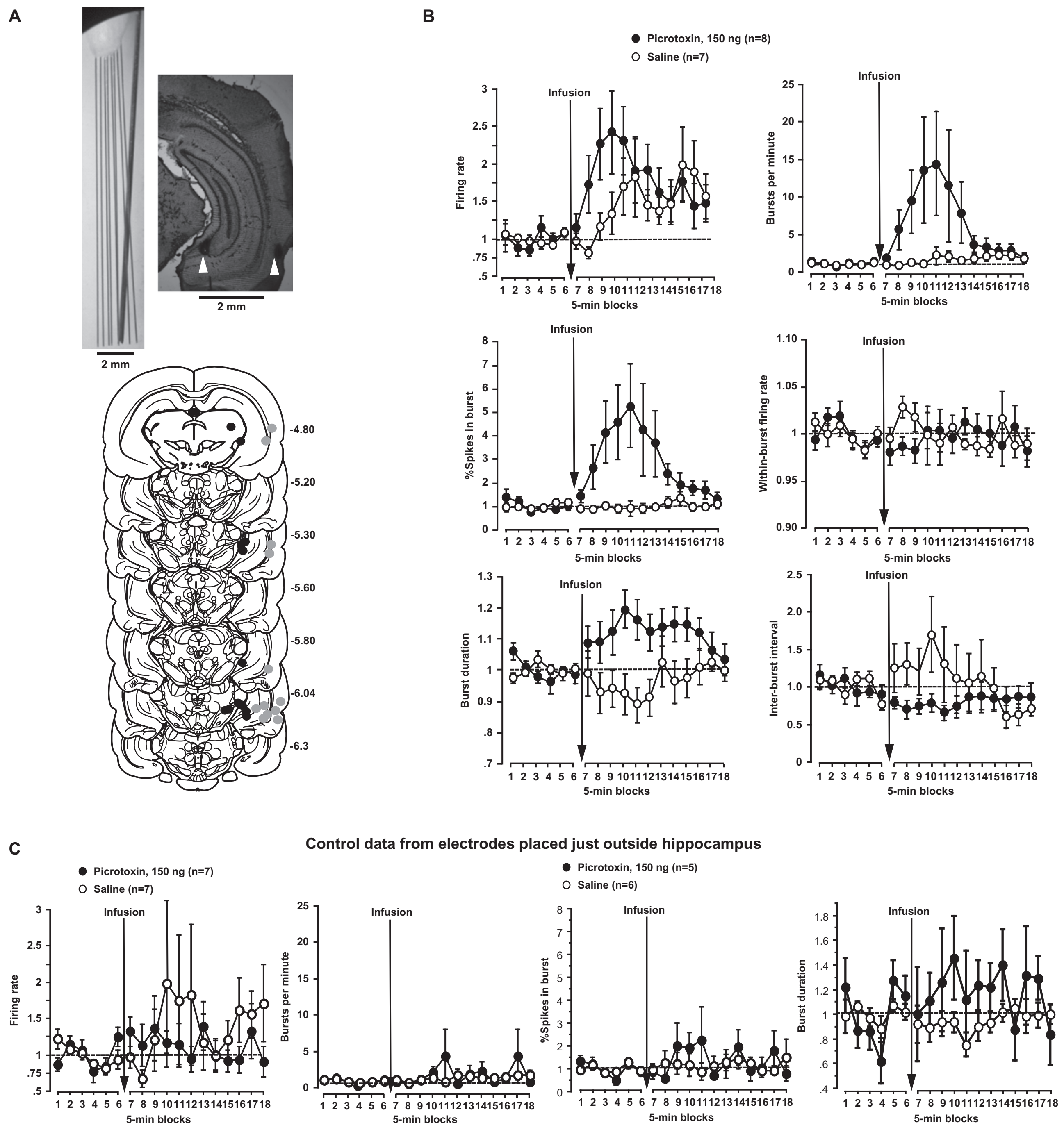

Figure 5. Picrotoxin increases overall firing rate and markedly enhances bursting within the hippocampus. (A) Placement of infusion-electrode array assembly: The photograph on the left shows the assembly of infusion cannula and 8-microwire-electrode array used to measure effects of drug microinfusions on hippocampal neural activity. The array was arranged perpendicular to the midline of the brain, with the infusion cannula located just posterior to the center of the array. The photograph on the right shows an exemplar coronal section through the hippocampus with markings (highlighted by white arrow heads) from the most medial and most lateral electrodes of the array. In this case, the 2 most laterally located electrodes were located outside of the hippocampus. Underneath the photographs, the approximate locations of markings from the most medial (black dots) and most lateral (gray dots) electrodes for all rats included in the electrophysiological studies are shown on coronal plates from Paxinos and Watson (1998), with numbers indicating distance from bregma in millimeters. Based on the locations of the markings, the most medial and/or the most lateral electrodes, typically 1–3 electrodes per rat, were located outside the hippocampus in all rats. Data recorded from electrodes placed outside hippocampus were analyzed separately from data recorded from the hippocampus, providing a control measure for drug spread outside the hippocampus. (B) Data recorded at electrodes placed within the hippocampus: Time courses of multiunit measures during baseline recordings and following infusion of picrotoxin or saline. All values are normalized to baseline (average of the 6 baseline 5-min blocks) and are presented as means ± SEM. The stippled horizontal line indicates baseline and the arrow indicates the time of infusion. (C) Data recorded at electrodes placed just outside hippocampus: Time courses of the multiunit measures (all values normalized to baseline, means ± SEM) that showed marked effects of picrotoxin infusion when recorded within hippocampus. Note absence of any clear picrotoxin effect, supporting that drug spread was largely restricted to within the hippocampus.

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Table 2. Preinfusion baseline values (mean ± SEM) of all electrophyisiological measures recorded from the hippocampal electrodes in the 2 prospective infusion groups

	Overall firing rate (1/s)	Bursts (1/min)	% Spikes fired in bursts	Within-burst firing rate	Burst duration (ms)	Interburst interval (s)	LFP AUC of PSD (μV^2)
Saline	42.1 ± 11.8	265.5 ± 75.5	28.8 ± 3.9	381.3 ± 6.1	10.1 ± 0.5	6.6 ± 1.7	0.0411 ± 0.0035
Picrotoxin (150 ng)	35.4 ± 3.4	251.11 ± 27.7	25.6 ± 1.6	401.8 ± 4.9	9.2 ± 0.2	4.4 ± 0.8	0.0303 ± 0.0025
Effect of infusion	$F_{1,13} < 1$	$F_{1,13} < 1$	$F_{1,13} < 1$	$F_{1,13} = 1.2$, $P = 0.3$	$F_{1,13} < 1$	$F_{1,13} < 1$	$F_{1,13} = 1.4$, $P = 0.3$

et al. 2009). Overall LFP power, measured as the AUC of PSD, was not affected by picrotoxin (main effect: $F_{1,13} < 1$; interaction infusion × 5-min block: $F_{17,221} = 1.23$; $P = 0.24$) (data not shown).

To support the spatial selectivity of the picrotoxin effects, we analyzed the data recorded from electrodes placed just outside the medial or lateral boundaries of the hippocampus (i.e., in the surrounding thalamus or parahippocampal cortex). In contrast to the marked picrotoxin-induced enhancement of burst firing within the hippocampus, as reflected by significant increases in “bursts per minute,” “percentage of spikes fired as bursts,” and “burst duration,” concomitant with a significant increase in overall firing rate (see above and Fig. 5B), picrotoxin did not affect these parameters at the misplaced electrodes just outside the hippocampus (all interactions and main effects involving the factor infusion, $P > 0.15$) (Fig. 5C). There are limitations to this analysis: 1) the number of misplaced electrodes was small; 2) in one rat receiving picrotoxin infusion, the only misplaced electrode was damaged, so that data of misplaced electrodes were only available from $n = 7$ rats in the picrotoxin group; 3) in some rats, none of the few misplaced electrodes recorded any bursts during the baseline period, so that these rats had to be excluded from the analysis of burst parameters normalized to baseline, resulting in $n = 5$ in the picrotoxin and $n = 6$ in the saline group; 4) electrodes were placed in 2 different regions (thalamus or parahippocampal cortex). These factors probably contributed to higher variability of the data recorded from the misplaced electrodes. Nevertheless, the control data from the misplaced electrodes support that picrotoxin effects were largely restricted to the hippocampus. This is consistent with autoradiographic studies showing that intrahippocampally infused radiolabelled AP-5, even at the relatively high infusion volume of 1 μL (in the present study, we used 0.5 μL), does not substantially spread across the medial and lateral boundaries of the hippocampus, probably because of the densely packed fiber bundles surrounding the hippocampus (Morris et al. 1989; Steele and Morris 1999).

Discussion

Hippocampal neural disinhibition by the GABA-A antagonist picrotoxin impaired attention, without affecting other behavioral measures, on the 5-CSRT test and caused deficits in rapid place learning performance on the watermaze DMP test. Moreover, hippocampal disinhibition moderately increased locomotor activity and slightly reduced startle reactivity, leaving PPI unaffected. In vivo electrophysiological recordings showed that picrotoxin mainly enhanced burst firing of hippocampal neurons.

Enhanced Burst Firing

Disinhibition by picrotoxin enhanced hippocampal burst firing and concomitantly increased overall firing rate in acute in vivo electrophysiological experiments under anesthesia. These findings are consistent with the enhanced hippocampal burst firing recently reported following pharmaco- or optogenetic silencing of hippocampal inhibitory interneurons in vitro (Lovett-Barron et al. 2012) and in awake mice (Royer et al. 2012), and corroborate the important role of GABAergic inhibition in hippocampal burst regulation. Aberrant burst firing may substantially interfere with cognitive functions of the hippocampus and its projection sites, as burst firing has been implicated in the encoding and readout of hippocampal memory (Takahashi and Magee 2009; Xu et al. 2012) and is particularly effective in driving postsynaptic targets (Lisman 1997). Our electrophysiological findings, in conjunction with our behavioral findings, suggest that aberrant hippocampal burst firing causes attentional and memory deficits.

Attentional Deficits

The selective attentional deficits on the 5-CSRT test, indicated by reduced accuracy without changes in other performance measures, probably reflect disruption of extrahippocampal processing, most likely in prefrontal cortex or ventral striatum through strong hippocampal functional connectivity to these sites (Bast 2011). Previous lesion studies suggest that the hippocampus itself plays, if at all, only a minor role in mediating sustained attention as measured on the 5-CSRT and related tests. On the 5-CSRT test, temporal hippocampal lesions (made after pretraining to criterion levels) mainly increased rats’ impulsive/premature responding, with little disruption of attentional performance, possibly as a side effect of increased impulsive responding, whereas septal (or dorsal) hippocampal lesions did not substantially affect task performance (Abela et al. 2013). Although one study reported persistent reductions in accuracy on the 5-CSRT test following lesions to the temporal hippocampus (made before pretraining), these were accompanied by large increases in perseverative responding, with the number of premature responses not reported (Le Pen et al. 2003). Consistent with hippocampal lesions disrupting aspects of inhibitory response control required on the 5-CSRT test, rather than sustained attention, complete hippocampal lesions did not affect attentional performance on a prefrontal-dependent self-paced serial-reaction task requiring little impulse control (Burk and Mair 2001). In contrast, sustained attention is highly dependent on balanced prefrontal activity, with prefrontal lesions (Chudasama and Robbins 2006), functional inhibition (by the GABA-A agonist muscimol), or disinhibition (Pezze et al. 2014) all markedly disrupting attention on the 5-CSRT test. Sustained attention on the 5-CSRT test also requires an optimal level of prefrontal (Granon et al. 2000) and ventral striatal (Pezze et al. 2007) dopamine receptor stimulation, which may be disrupted by hippocampal neural disinhibition, given that hippocampal stimulation activates the meso-prefrontal-ventral striatal dopamine system (Mitchell et al. 2000; Floresco et al. 2001; Peleg-Raibstein et al. 2005; Bast 2011; Lodge and Grace 2011). Future studies will have to clarify the extrahippocampal,

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including prefrontal and ventral striatal, neural effects of hippocampal disinhibition. The attentional deficits following hippocampal neural disinhibition highlight that the cognitive impact of regional neural disinhibition can extend beyond functions normally depending on the disinhibited region (see also Auger and Floresco 2015).

Contrasting with the selective reduction in accuracy following hippocampal neural disinhibition in the present study, attentional deficits on the 5-CSRT test following lesions (Chudasama and Robbins 2006), as well as functional inhibition or disinhibition (Pezze et al. 2014), of the medial prefrontal cortex manifest as reduced accuracy alongside increased omissions (additionally, lesions and functional inhibition affect response control measures). However, experimental manipulations primarily targeting the afferent modulation of the prefrontal cortex have been reported to cause selective reductions in accuracy without increasing omissions, including selective manipulations of the cholinergic (McGaughy et al. 2002) or dopaminergic (Granon et al. 2000) modulation of the prefrontal cortex. Selective reductions in accuracy, without increases in omissions, have also been reported in the triple transgenic mouse model of Alzheimer's disease (Romberg et al. 2011) and the pilocarpine rat model of temporal lobe epilepsy (Faure et al. 2014), where the primary pathology is not within the prefrontal cortex, but in brain regions, including medial temporal lobe regions, that may modulate the prefrontal cortex. It is entirely consistent with these studies that hippocampal neural disinhibition, which increases hippocampal burst firing and, by way of hippocampo-prefrontal functional connectivity, would modulate prefrontal function, causes similar selective reductions in accuracy on the 5-CSRT task. Interestingly, both the pilocarpine rat model (Kumar and Buckmaster 2006) and the triple transgenic mouse model of Alzheimer's disease (Davis et al. 2014) show hippocampal hyperexcitability; our new finding that hippocampal neural disinhibition causes attentional impairments suggests that hippocampal hyperexcitability may contribute to the attentional deficits in these rodent models.

Memory Deficits

Hippocampal picrotoxin markedly disrupted rapid place learning performance on the watermaze DMP test, as reflected by a marked reduction of search preference for the new location, which rats had to learn within the first trial of the day. Previous studies suggest that performance on the watermaze DMP test depends on the hippocampus for the required rapid encoding of new places and for the translation of such rapid spatial updating into behavioral performance. The DMP test is disrupted by pharmacological manipulations targeting synaptic plasticity mechanisms mediated by NMDA (Steele and Morris 1999) and dopamine receptors (Pezze and Bast 2012) and by partial hippocampal lesions, including lesions restricted to temporal and intermediate hippocampus (Bast et al. 2009), which were targeted by the infusions in the present study. Functional inhibition targeting the intermediate hippocampus also disrupts task performance (McGarrity et al. 2014). The requirement of temporal to intermediate hippocampus probably reflects that these regions feature functional connectivity to frontal and subcortical sites necessary to translate hippocampal learning into performance, although the specific relevant brain sites remain to be determined (Bast et al. 2009; Bast 2011). Therefore, neural disinhibition, causing aberrant neuronal bursting, may disrupt DMP performance by interfering with hippocampal encoding or readout of relevant place information or with the passing on of such information to hippocampal projection sites.

Our finding, highlighting the importance of GABAergic inhibition for hippocampus-dependent memory performance, converges with recent studies in mice reporting learning-related increase of hippocampal inhibitory synapses (Ruediger et al. 2012) and impaired memory performance following disruption of hippocampal GABA neuron function by molecular-, opto- or pharmacogenetic approaches (Prut et al. 2010; Murray et al. 2011; Andrews-Zwilling et al. 2012; Caputi et al. 2012; Donato et al. 2013; Gilani et al. 2014; Lovett-Barron et al. 2014; Engin et al. 2015; Lee et al. 2016). Moreover, our findings support recent studies in humans and rodent models linking hippocampal overactivity and hyperexcitability to age-related memory deficits (Koh et al. 2010; Bakker et al. 2012; Davis et al. 2014) and are consistent with the correlation of hippocampal overactivity with memory deficits in schizophrenia (Tregellas et al. 2014). However, hippocampal neural disinhibition may facilitate hippocampal synaptic plasticity and, thereby, improve memory, if such disinhibition is finely and dynamically regulated by endogenous plasticity (Donato et al. 2013) or if there is a pre-existing deficit due to increased neural inhibition (Fernandez et al. 2007). Moreover, systemic injection of a selective inverse agonist to negatively modulate GABA-A receptors containing the α5 subunit, which are predominantly expressed in hippocampus and constitute about 20% of hippocampal GABA-A receptors, has been suggested to facilitate hippocampal plasticity and memory (Dawson et al. 2006), although transgenic reduction of α5 subunit-containing GABA-A receptor expression in the hippocampus has also been reported to disrupt aspects of hippocampus-dependent memory (Prut et al. 2010; Engin et al. 2015). Interestingly, a recent study suggests that enhancing the amplitude, but not duration, of synaptic excitation within the prefrontal cortex (by optogenetic stimulation of glutamatergic neurons or local infusion of a specific AMPAkine) enhances prefrontal-dependent recognition memory (Benn et al. 2016). Similarly, the selective inverse agonist at α5 subunit-containing GABA receptors may enhance hippocampus-dependent memory by enhancing the amplitude of hippocampal neural activity, while leaving the temporal pattern of neural activity largely unaffected (this is consistent with in vitro evoked potential findings that the drug enhances LTP induction, without affecting stimulation-evoked field potential bursting; Dawson et al. 2006). In contrast, hippocampal neural disinhibition caused by picrotoxin in the present study altered the temporal organization of hippocampal neural activity (enhancing burst-pattern firing). Overall, hippocampus-dependent memory performance appears to require hippocampal neural activity that is appropriately balanced by GABAergic inhibition, resembling the requirement of appropriately tuned prefrontal activity for prefrontal-dependent cognitive functions (Gruber et al. 2010; Pezze et al. 2014; Tse et al. 2015).

Sensorimotor Effects

Locomotor hyperactivity and reduced startle reactivity caused by hippocampal picrotoxin in Lister hooded rats in the present study replicate findings in Wistar rats (Bast et al. 2001a). These moderate effects are unlikely to have interfered with performance on the cognitive tests, which is supported by normal response and reward-collection latencies. Locomotor hyperactivity is consistent with the idea that increased hippocampal neuron activity drives the dopamine system and associated behavioral changes, which may be relevant to psychosis

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(Mitchell et al. 2000; Floresco et al. 2001; Bast and Feldon 2003; Bast 2011; Lodge and Grace 2011; Gilani et al. 2014).

Hippocampal picrotoxin did not affect PPI in Lister hooded rats, contrasting with the marked PPI disruption in Wistar rats (Bast et al. 2001a). Considered together with our recent finding that prefrontal picrotoxin, which markedly disrupts PPI in Sprague Dawley rats (Japha and Koch 1999), does not affect PPI in Lister hooded rats (Pezze et al. 2014), this suggests that the forebrain modulation of PPI is less pronounced in Lister hooded than in other rat strains, adding to strain and species differences in PPI modulation (Swerdlow et al. 2008).

Cognitive and Behavioral Impact of Neural Disinhibition: Comparison to Inactivation and Lesions

The findings that hippocampal neural disinhibition disrupted hippocampus-dependent place learning performance on the watermaze DMP task (similar to hippocampal lesions and inactivation) and attentional performance on the 5-CSRT test (similar to prefrontal lesion, inactivation and disinhibition) supports the idea that hippocampal neural disinhibition, by disrupting balanced neural activity locally within the hippocampus or distally within hippocampal projection sites, may disrupt cognitive and behavioral functions depending on the hippocampus and its projection sites, such as the medial prefrontal cortex (Bast 2011).

However, other hippocampus- and prefrontal-dependent functions can be largely unaffected by hippocampal neural disinhibition. In the present study, hippocampal neural disinhibition did not affect response control on the 5-CSRT test (as reflected by unchanged premature or perseverative responses), which has been shown to be markedly disrupted by both prefrontal and hippocampal lesions and/or inactivation (Chudasama and Robbins 2006; Chudasama et al. 2012; Abela et al. 2013; Pezze et al. 2014); similar to hippocampal neural disinhibition, prefrontal disinhibition also does not affect response control (Pezze et al. 2014). This suggests that response control requires neural activity within the hippocampo-prefrontal circuit, but not the appropriate tuning of such activity. In other words, response control can be sustained as long as neural activity within the hippocampo-prefrontal circuit is above a minimal level.

Moreover, neural disinhibition of the (temporal) hippocampus (similar to direct chemical or electrical stimulation, see Bast and Feldon, 2003) enhances locomotor activity (present study; Bast et al. 2001a), which depends on neural activity within the temporal hippocampus and is reduced by inactivation of this region (Bast et al. 2001b). Similarly, prefrontal neural disinhibition increases, whereas prefrontal functional inhibition decreases locomotor activity (Pezze et al. 2014). These findings suggest that neural activity within hippocampus and prefrontal cortex drives open-field locomotor activity, with a monotonic positive relation between neural activity in these areas and locomotion. These locomotor effects likely reflect a positive modulation of ventral striatal dopamine transmission by neural activity within the hippocampo-prefrontal circuit (Karreman and Moghaddam 1996; Mitchell et al. 2000; Floresco et al. 2001; Bast and Feldon 2003; Lodge and Grace 2011).

Overall, regional neural disinhibition can impact on cognitive and behavioral functions mediated by the disinhibited region and by distal brain sites with which the disinhibited region is functionally connected. Depending on the specific hippocampus- or prefrontal-dependent function, hippocampal neural disinhibition may 1) have a disruptive effect (i.e., have similar effects to hippocampal and/ or prefrontal lesions or inactivation), 2) have no effect, or 3) enhance the function (i.e., have opposite effects to hippocampal or prefrontal inactivation or lesion). These different effects suggest that different hippocampus- and prefrontal-dependent functions show different relationships to neural activity within hippocampus and prefrontal cortex. In addition, the impact of hippocampal neural disinhibition can extend beyond hippocampus- and prefrontal-dependent functions, reflecting, for example, hippocampal functional links to subcortical sites (e.g., impact on subcortical dopamine transmission that is thought to drive locomotor activity).

Clinical Relevance

Hippocampal GABA dysfunction has been implicated in schizophrenia and age-related cognitive decline (Lisman et al. 2008; Huang and Mucke 2012; Stanley et al. 2012; Heckers and Konradi 2015; Nava-Mesa et al. 2014; Ruzicka et al. 2015). Given the close link between neural activity and metabolic activation (Sokoloff 1981), the enhanced hippocampal multiunit activity caused by acute GABA antagonism is consistent with the hippocampal metabolic overactivity characterizing early stages of these disorders (Schobel et al. 2009; Bakker et al. 2012; Huang and Mucke 2012). Compensatory adjustments and excitotoxicity associated with long-term GABA dysfunction and neural overactivity may contribute to regional hypoactivity and atrophy characterizing later disease stages (Huang and Mucke 2012; Schobel et al. 2013; Anticevic et al. 2015), aspects of these chronic disorders not mimicked by acute GABA antagonism.

The 5-CSRT and DMP tests have high validity to measure deficits in attention and memory relevant to clinical disorders, with related human paradigms—continuous performance tests and place learning tests in analogs of the watermaze, respectively—revealing marked deficits in schizophrenia and age-related cognitive decline (Chudasama and Robbins 2006; Hort et al. 2007; Lustig et al. 2013; Romberg et al. 2013; Fajnerova et al. 2014). Our present findings in rats suggest that hippocampal neural disinhibition contributes to clinically relevant attentional and memory deficits. Furthermore, they support that causal relationships underly the recently reported correlations of hippocampal overactivity with both memory and attentional deficits in schizophrenia patients (Tregellas et al. 2014) and the association of hippocampal overactivity with memory deficits in amnestic mild cognitive impairment (Bakker et al., 2012).

Conclusions

Hippocampal neural disinhibition causes dysregulation of local neuron firing characterized by enhanced bursting, which would be expected to disrupt hippocampal processing and cause aberrant drive to hippocampal projection sites. Consistently, hippocampal disinhibition disrupts hippocampus-dependent rapid place learning performance, as well as aspects of attentional performance that do not normally require the hippocampus, but are mediated by prefrontal-striatal mechanisms. The latter supports that hippocampal dysfunction may partly manifest through deficits in prefrontal-dependent function, consistent with strong hippocampo-prefrontal functional connectivity (Meyer-Lindenberg et al. 2005; Bast 2011). The attentional and memory deficits caused by hippocampal neural disinhibition, together with findings that prefrontal-cortical disinhibition disrupts attentional and executive functions (Gruber et al. 2010; Enomoto et al. 2011; Paine et al. 2011, 2015; Pehrson et al. 2013; Pezze et al. 2014; Tse et al. 2015), highlight the importance of

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cortico-hippocampal GABAergic inhibition for cognitive function. This supports that cortico-hippocampal neural disinhibition, which is a key feature of schizophrenia (Lisman et al. 2008; Heckers and Konradi 2015; Tse et al. 2015) and has been implicated in other disorders, most notably age-related cognitive decline (Huang and Mucke 2012; Stanley et al. 2012; Nava-Mesa et al. 2014), contributes to causing key cognitive deficits characterizing these disorders and, hence, is an important treatment target.

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