Partial Disinhibition Is Required for Transition of Stimulus-Induced Sharp Wave–Ripple Complexes Into Recurrent Epileptiform Discharges in Rat Hippocampal Slices

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Hippocampal sharp waves consist of slow field potential transients that are superimposed by approximately 200-Hz network oscillations, termed “ripples” (Chrobak et al. 2000), and are predominantly observed in vivo during consummatory behavior and slow-wave sleep (Buzsáki 1986, 1998). Similar network activity has been observed in vitro where spontaneous sharp wave–ripple complexes (SPW-Rs) have been recorded in rodent hippocampal slices (Behrens et al. 2005; Both et al. 2008; Maier et al. 2003; Nimmrich et al. 2005). We have previously shown that in rat hippocampal slices SPW-Rs can be induced by standard protocols for induction of late or long-lasting long-term potentiation (LTP) (Behrens et al. 2005; Frey and Morris 1997). This is reminiscent of kindling-induced epileptiform activity. Interestingly, SPW-R-like activity has also been observed in epileptic rodents and in humans, suggesting that these events may serve as a biomarker for an epileptogenic zone (Staba et al. 2004). Indeed, it was suggested that stimulus-induced SPW-Rs are a model of epilepsy (Staley and Dudek 2006). We therefore decided to compare the properties of SPW-Rs and epileptiform activity induced by partial removal of inhibition and furthermore studied conditions under which SPW-Rs were converted into recurrent epileptiform discharges. In the present study, we investigated the effects of increased extracellular potassium concentration ([K+]) on induced SPW-R activity and compared these with the effects of increased extracellular potassium concentration ([K+]o, 8.5 mM).

The hippocampus is densely packed with a variety of nicotinic acetylcholine receptors (nAChRs) localized on both principal cells and interneurons (Albuquerque et al. 1995; Freund and Katona 2007; Ji et al. 2001). Nicotine influences synaptic transmission in hippocampal slices (Giocomo and Hasselmo 2005; Radcliffe et al. 1999) and an α7-nAChR-dependent reduction of the GABAergic inhibition has been shown in area CA1 (Zhang and Berg 2007). We showed that nicotine, which in contrast to recent observations in cerebellar slices, did not induce synchronized network discharges in area CA3 of naïve slices, but dose-dependently transformed SPW-Rs into prolonged network discharges reminiscent of REDs. This was associated with a partial reduction in the inhibitory conductance in CA3 pyramidal cells. Similarly, a dose-dependent transition of SPW-Rs into REDs resulted from application of BMI, whereas positive modulation of γ-aminobutyric acid type A (GABA_A)–mediated inhibition by phenobarbital significantly diminished SPW-R activity. Elevating [K+]o has been shown to induce burst-like discharges in hippocampal pyramidal cells (Jensen et al. 1994; Korn et al. 1987; Ruttecki et al. 1985) and to augment spontaneous and action potential (AP)–dependent transmitter release (Hablitz and Lundervold 1981), in part, by slowing repolarization of APs (for review see Lux...
were tested while in the aCSF (Whittington et al. 1995), we investigated whether MgSO_4 1.8, NaH_2PO_4 1.25, and glucose 10, saturated with 95% absence of glutamatergic transmission, inhibitory conductance of stimulus-induced SPW-Rs were increased, whereas notably, the propensity of elevated [K^+]_o to induce seizure-like events (SLEs) was higher in naïve slices compared with stimulated slices, which expressed SPW-Rs. Together, our in vitro findings indicate that reduced GABA_A-mediated inhibition is one key prerequisite needed to transform SPW-Rs into recurrent epileptiform discharges.

METHODS

Slice preparation

Animal procedures were performed in accordance with the guidelines of the European Communities Council and approved by the regional authority (LaGeSo Berlin: T0068/02). Wistar rats (aged 6–8 wk, ~200 g) of either sex were decapitated under deep ether anesthesia. Horizontal hippocampal slices (400 μm at bregma ~4.7 to ~7.3 mm) were prepared at an angle of about 12° in the frontocircumical direction (with the frontal portion up) using a vibratome (752 M Vibrorslice; Campden Instruments, Loughborough, UK). Preparation of slices was done in ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl 129, NaHCO_3 21, KCl 3, CaCl_2 1.6, MgSO_4 1.8, NaH_2PO_4 1.25, and glucose 10, saturated with 95% O_2–5% CO_2. Slices were immediately transferred to an interface chamber perfused with aCSF at 36 ± 0.5°C (flow rate: ~1.6 ml/min, pH 7.4, osmolality: 295–300 mosmol/kg). Recordings were started 2–3 h after preparation. Except for experiments concerning monosynaptic inhibitory postsynaptic potentials (IPSPs), the concentration of MgSO_4 was lowered to 1.2 mM (a value close to physiological Mg^2+ concentrations; Windmuller et al. 2005) 1 h before starting the recordings for all experiments. Since a reduction of the IPSP conductance has been described during prolonged epileptiform activity on lowering of extracellular magnesium concentration [Mg^{2+}]_o to 0 mM in the aCSF (Whittington et al. 1995), we investigated whether lowering of [Mg^{2+}]_o from 1.8 to 1.2 mM in aCSF resulted in changes in GABAergic inhibition. Control experiments were performed in CA3 pyramidal cells, where changes in GABA_A-mediated inhibition were tested while [Mg^{2+}]_o was lowered from 1.8 to 1.2 mM in aCSF. This had no significant effect on the conductance of IPSPs, which was slightly but not significantly reduced (Ctl: 25.2 ± 3.6 nS; after 15 min: 22.6 ± 3.6 nS; after 30 min; 26.1 ± 1.2 nS; and after 45 min: 24.7 ± 2.4 nS, P > 0.05, n = 5; data not shown).

Electrophysiological recordings

Extracellular field potentials (FPs) were recorded under interface conditions using a custom-made amplifier from the stratum pyramidale of area CA3, with microelectrodes filled with 154 mM NaCl (5–10 MΩ). For intracellular recordings, sharp microelectrodes (70–90 μm) were pulled from borosilicate glass (OD 1.2 mm) and filled with 2.5 M K^+ -acetate. The sharp microelectrode technique was chosen because it allows stable recordings demanded for long-term intracellular recordings during SPW-Rs. Extra- and intracellular signals were amplified by a SEC 05L amplifier (NI Electronic Instruments, Tamm, Germany). All data were low-pass filtered at 3 kHz, digitized at 10 kHz, and stored on computer disk using a CED 1401 interface (Cambridge Electronic Design [CED], Cambridge, UK). Intracellular recordings were accepted when membrane potentials of the cells were < −60 mV, action potentials (APs) exceeded 75 mV, and input resistance was >25 MΩ. Input resistance was determined by hyperpolarizing current injection pulses of 0.2–0.4 nA for 0.2–0.6 s.

To measure changes in [K^+]_o, we used double-barreled K^+-sensitive microelectrodes for DC-coupled recordings in area CA3. K^+-sensitive microelectrodes were manufactured as previously described (Heinemann and Arens 1992). In brief, K^+-sensitive microelectrodes were tip-filled with the potassium ionophore cocktail A (60031; Fluka Chemie, Buchs, Switzerland) and accepted when they responded with 59 ± 1 mV to a change in [K^+]_o from 3 to 30 mM.

In recordings where we tested for effects mediated by nicotine, bicuculline, or elevation of [K^+]_o from 3 to 8.5 mM on GABAergic inhibition onto CA3 pyramidal cells (APV (50 μM) was coapplied with 6-cyano-7-nitroquinazoline-2,3-dione (CNQX, 25 μM), to block glutamatergic synaptic transmission. To study evoked IPSPs, depolarizing and hyperpolarizing current steps (0.06 to −0.12 nA of 0.5-s duration; interval: ~1.7 s) were applied. An extracellular single pulse stimulation at 1 Hz, 0.5 ms, 4 V (tip potential of cell response) was applied with a delay of 0.25 s to the onset of each current pulse. Monosynaptic IPSPs were evoked by stimulation of either stratum radiatum (SR) or stratum oriens (SO) of area CA3 (see Davies and Collingridge 1993; Romo-Parra et al. 2008).

Induction of SPW-Rs

SPW-Rs were induced by high-frequency stimulation (HFS, containing three tetani of 40 pulses applied at 100 Hz, each; pulse duration: 0.1 ms; interspike interval: 40 s; HFS was repeated every 5 min) using a bipolar platinum electrode (25 μm, tip separation: 100–150 μm) placed in the SR of area CA1. Slices were stimulated using submaximal (70%) stimulus intensity (1.5–3 V) of that required to evoke maximal amplitude field responses.

Data analysis

To analyze different components of the SPW-Rs and REDs, raw data were filtered using the digital filter function in Spike2 software (CED) as previously described (Behrens et al. 2005). In brief, for ripple detection, we used a band-pass filter of 40–400 Hz with a threshold set to 4–6 SD of eventless baseline noise. For sharp wave detection, recordings were low-pass filtered at 20 Hz (Spike2 software). Ripple frequency was determined using custom-made software (H. Siegmund).

In the experiments where we tested for the modulation of the GABAergic inhibition onto CA3 pyramidal cells, changes in the amplitude of evoked IPSPs were measured during positive and negative current steps. Because sharp microelectrodes were used for long-term recordings of single-cell responses during SPW-Rs, we also used this method to calculate the membrane conductance. To determine the effects on synaptic conductance, a linear regression of the evoked potentials as a function of depolarizing and hyperpolarizing current injection was calculated using Origin software (Version 6, Microcal Software, Northampton, MA). For each condition (control, 100 and 500 μM nicotine, 1 and 2 μM BMI, [K^+]_o, 8.5 mM) the slope ± SD was calculated. The IPSP conductance was estimated by regression analysis from the plotted slope of the relation between the membrane potential deflection at the peak of the IPSPs versus injected current, minus the resting conductance of the cell (Luhmann and Prince, 1991). For statistical comparison, absolute data, obtained from each experiment, were normalized. To analyze changes in cellular timing during ripple oscillations, we measured the jitter between a given field ripple and the corresponding intracellular recorded AP. For that purpose, we measured the latency between the negative peak of the field ripple and the positive peak of a given AP. The calculated jitter was determined as the SD of the measured mean latencies between ripples and APs under defined conditions (control, 100 and 500 μM).
nicotine). For comparison, changes in the jitter were normalized. All data are reported as means ± SE. Statistical significance was determined using one-way ANOVA (Origin 6.0; Microcal); $P < 0.05$ (*) was considered to indicate a significant difference.

**Drugs**

All drugs were dissolved in aCSF and applied by continuous bath perfusion. Nicotine tartrate (100 and 500 μM) was applied for 25–40 min; methyllycaconitine citrate hydrate (MLA, a specific α7 receptor antagonist, 10 nM) and mecamylamine hydrochloride (MEC, an unspecific nAChR antagonist, 25 μM) were used to test for specificity of nicotine effects. To mimic the effects of nicotine by acetylcholine (ACh) we used acetylcholine chloride (10 μM) in presence of atropine (1 μM) and physostigmine (2 μM). To investigate the effects of nicotine, BMI, or elevation in $[K^+]_o$, 8.5 mM on evoked monosynaptic IPSPs on pyramidal cells, dl-2-amino-5-phosphonopentanoic acid (DL-APV sodium salt, 50 μM) and 6-cyano-7-nitroquinolinedione-2,3-dione (CNQX disodium salt, 25 μM) were applied to block the glutamatergic transmission. To investigate the α7-nAChR specificity of nicotine-mediated effects on evoked IPSPs, we added the α7-nAChR antagonist MLA to the bath solution, including DL-APV and CNQX prior to the application of nicotine. Bicuculline methiodide (BML, 1–3 μM) was applied to determine which amount of fractional loss of inhibition was required for transformation of SPW-Rs into REDs and to verify that evoked IPSPs were GABA$\scriptsize{\text{A}}$-dependent. Sodium phenobarbital (20 μM, courtesy of Dr. Holtkamp) was applied to test effects of increases in inhibition on SPW-R activity by modulation of GABA$\scriptsize{\text{A}}$ receptors. All drugs were purchased from Sigma-Aldrich (Taufkirchen, Germany), except DL-APV and CNQX (Ascent Scientific, Bristol, UK).

**RESULTS**

**Nicotine-mediated effects on area CA3 in naïve slices**

Nicotine has recently been shown to evoke synchronized network oscillations in the cerebellum (Middleton et al. 2008). To test whether in hippocampal area CA3 nicotine application resulted in the induction of synchronized network activity, nicotine was bath applied in a concentration of 100 or 500 μM for 45 min to unstimulated, naïve slices. Extracellular field potential (FP) recordings obtained from the stratum pyramidale in area CA3 revealed that neither 100 μM (n = 6 slices) nor 500 μM of nicotine (n = 5 slices) induced synchronized network activity (data not shown). To investigate whether nicotine exerted effects on stimulus-evoked FPs in area CA3, single-pulse stimulation was applied to the stratum radiatum (SR) in area CA1 (Fig. 1A), thereby activating Schaffer collaterals, which originate from CA3 pyramidal cells. This stimulation induced antidromic FP responses in area CA3, which consisted of two population spikes (PSs): a first antidromic PS due to direct activation of CA3 pyramidal cell axons and a second PS, representing the population response generated by transmission via short, recurrent axon collaterals onto neighboring CA3 cells within the associational CA3 network (see Fig. 1B). We found that nicotine applied in 100 and 500 μM did not significantly affect the antidromically evoked first PS (Fig. 1C). In contrast, the second PS was significantly and reversibly enhanced to 162.7 ± 8.5% ($P < 0.004$) and to 179.8 ± 11.0% of control ($P < 0.001$) when nicotine was applied at 100 and 500 μM, respectively (washout: 102.4 ± 15.1%, $n = 6$ slices, $P > 0.05$; Fig. 1, C and D). In addition, in the presence of 500 μM nicotine, we observed the occurrence of multiple recurrent PSs (Fig. 1C), suggesting a loss of inhibition.

**Nicotine facilitates stimulus-dependent induction of SPW-Rs and modulates their properties**

As previously reported, hippocampal sharp wave–ripple complexes (SPW-Rs) could be induced by repeated high-frequency stimulation (HFS), which reliably induced long-term potentiation (LTP) (Behrens et al. 2005). Simultaneous triple FP recordings obtained from stratum pyramidale in area CA3 and CA1 and stratum granulare in the dentate gyrus (DG) revealed that stimulus-induced SPW-Rs originated in the CA3 region, whereas they orthodromically spread into the CA1 region as previously shown (Behrens et al. 2005). In dentate gyrus, rather small negative potentials were observed. Such events presented with amplitudes <150–200 μV, and usually followed events in area CA3, whereas they occurred relatively simultaneously with CA1 events where transients were negative in SR neighboring the DG. These events may therefore represent far field effects or a back-propagation from CA3 to the dentate gyrus (Scharfman 2007). We did not further analyze these oscillations in the present study (data not shown).

Since the cholinergic agonist nicotine is known to facilitate induction of LTP in various brain regions including the hippocampal formation (Fujii et al. 2000; Nashmi and Lester 2006; Séguela et al. 1993), we first investigated whether nicotine showed any effects on the induction of SPW-Rs (Supplemental Fig. S1). In this set of experiments, nicotine was regularly administered 30 min before HFS was started (HFS consisted of three short tetani applied at 100 Hz; see METHODS). Under control conditions, SPW-Rs appeared after 5.8 ± 0.4 tetanic stimulations ($n = 6$ slices), whereas in the presence of nicotine (100 and 500 μM) the threshold for
induction of SPW-Rs was reduced (Supplemental Fig. S1). In the presence of 100 μM nicotine, the number of tetani required to induce SPW-Rs was 3.0 ± 0.0 (n = 6 slices; P < 0.0001, Supplemental Fig. S1, B and E). The number of tetani required to induce SPW-R further decreased to 2.0 ± 0.7 when slices were pretreated with 500 μM nicotine (n = 6 slices; P < 0.0001, Supplemental Fig. S1, C and E). As shown in Supplemental Fig. S1D, prior application of the α7-NAC receptor antagonist methyllycaconitine (MLA, 10 nM) prevented the effect of 100 μM nicotine (n = 6; Supplemental Fig. S1, D and E). Importantly, in the absence of any stimulation, 500 μM nicotine did not cause any kind of spontaneous synchronized epileptiform discharges (data not shown).

To test potential nicotine-mediated effects on established SPW-Rs, HFS was repeated every 5 min as previously reported (Behrens et al. 2005). Following the sixth HFS, when stable SPW-Rs were induced, they occurred with an incidence of 12.2 ± 0.3 events/min and showed an average amplitude of 2.7 ± 0.1 mV (Fig. 2, Aa–Ac). SPW-Rs lasted on average for 54.8 ± 0.3 ms (n = 11 slices, Fig. 2, Ab and Ac). Ripple oscillations, which were superimposed on sharp waves, showed a mean frequency of 181.1 ± 2.0 Hz (n = 11 slices; Fig. 2Ac). To test whether nicotine affected stimulus-induced SPW-Rs, the drug was applied for 30–40 min during ongoing SPW-R activity. Nicotine applied in a concentration of 10 and 50 μM had no significant effects on the incidence, amplitude, or duration of established SPW-Rs (n = 6, P > 0.05, each; data not shown). In the presence of 100 μM nicotine, the incidence of SPW-Rs was slightly but not significantly increased to 13.5 ± 1.1 SPW-Rs/min (n = 5 slices, Fig. 2, Aa and Ac; washout: 5.3 ± 0.9 SPW-Rs/min). In contrast, 100 μM of nicotine caused a significant and reversible increase of the SPW-Rs amplitude to 4.1 ± 0.5 mV (n = 5 slices, P < 0.005, Fig. 2, Aa–Ac; washout: 2.3 ± 0.2 mV) with a duration of 56.1 ± 3.6 ms (n = 11 slices, Fig. 2, Ab and Ac). In this concentration, nicotine did not significantly change the frequency of ripple oscillations, which was 183.2 ± 4.8 Hz (n = 5 slices, Fig. 2Ac; washout: 185.5 ± 5.0 Hz).

When nicotine was applied at a concentration of 500 μM (n = 6 slices) SPW-Rs were transformed into recurrent epileptiform discharges (REDs), which occurred with an incidence of 9.2 ± 1.0 REDs/min (P < 0.02, Fig. 2, Ba and Bc; washout: 9.1 ± 0.7 REDs/min). REDs showed an amplitude of 5.4 ± 0.7 mV, which was significantly larger than that of control SPW-Rs (P < 0.001, washout: 3.1 ± 0.5 mV) and lasted on average 117.4 ± 11.3 ms (P < 0.001, Fig. 2, Ba–Bc; washout: 45.8 ± 5.9 ms). Under this condition, the ripple frequency was significantly increased to 210.8 ± 5.0 Hz (P < 0.04, Fig. 2Bc; washout 183.9 ± 3.6 Hz; the transition from SPW-R to REDs induced by 500 μM nicotine is shown later in Fig. 6). Usually, this transition began 20 min after onset of application, as indicated by an increase in amplitude, first, and subsequently the duration of single events, and was nearly complete after 30 min. As a measure for the underlying network activation, we analyzed changes in the extracellular potassium concentration ([K⁺]o) accompanying SPW-Rs, before and after application of 100 and 500 μM of nicotine since pronounced increases in [K⁺]o are characteristic for REDs both in vivo and in vitro (Behrens et al. 2007; Futamachi and Pedley 1976; Heinemann et al. 1977). As illustrated in Fig. 2C, 100 μM nicotine caused a pronounced increase in [K⁺]o during 500 μM nicotine application. A representative sample of increases in [K⁺]o (top) accompanying SPW-Rs (bottom) under control condition (left) and during application of 100 μM nicotine (right). Note massive increases in [K⁺]o during 500 μM nicotine-induced REDs. Cb: plot summarizing [K⁺]o increases under different conditions according to Ca (n = 5 slices, each, P < 0.05).

FIG. 2. Effects of nicotine on stimulus-induced sharp wave–ripple complexes (SPW-Rs). As: sample recording of 100 μM nicotine-mediated effects on established SPW-Rs. Left: control. Right: 100 μM nicotine; scale bars: 5 mV, 10 s. Ab: overlay of 10 samples of SPW-Rs before (left) and during application of 100 μM nicotine (right); scale bars: 5 mV, 0.1 s. Ac: plots summarizing 100 μM nicotine-mediated effects on SPW-Rs. Note that 100 μM nicotine caused an increase only in amplitude without major effects on ripple frequency, SPW-R incidence, and duration. Bc: analogous to Aa–Ac. Sample recording of 500 μM nicotine-mediated effects on SPW-Rs (left) control; Right: 500 μM nicotine; scale bars: 5 mV, 10 s. Bb: overlay of 10 samples of SPW-Rs before (left) and during application of 500 μM nicotine (right); scale bars: 5 mV, 0.1 s. Each. Bc: plots summarizing the effects of 500 μM nicotine on SPW-Rs. Note that 500 μM nicotine markedly prolonged SPW-Rs, transforming them into recurrent epileptiform discharges (REDs; *P < 0.05). Ca: representative samples of increases in [K⁺]o (top) accompanying SPW-Rs (bottom) under control condition (left) and during application of 100 μM nicotine (middle) and 500 μM nicotine (left). Note massive increases in [K⁺]o, during 500 μM nicotine-induced REDs. Cb: plot summarizing [K⁺]o increases under different conditions according to Ca (n = 5 slices, each, P < 0.05).
μM nicotine elevated the increases in [K+]₀ from 0.09 ± 0.01 to 0.22 ± 0.04 mM (n = 5, P < 0.05), whereas 500 μM of nicotine caused an elevation of increases in [K+]₀ to 1.12 ± 0.14 mM (n = 5, P < 0.001; Fig. 2C).

To test whether ACh application mimicked nicotine-mediated effects on SPW-Rs, we applied 10 μM ACh in the presence of atropine (1 μM) to block muscarinic receptors. Selective activation of nAChR by ACh during blocked muscarinic transmission did not cause significant changes in the properties of SPW-Rs (n = 4; see Suplemental Fig. S2, A and B; amplitude: control: 2.8 ± 0.3 mV, wash: 2.7 ± 0.5 mV, washout: 2.4 ± 0.65 mV; duration: control: 54.0 ± 2.0 ms, wash: in: 53.0 ± 3.1 ms, washout: 54.0 ± 2.0 ms; incidence: control: 13.1 ± 3.2 SPW-Rs/min, wash: in: 8.9 ± 2.3 SPW-Rs/min, washout: 7.5 ± 2.1 SPW-Rs/min; ripple frequency: control: 197.5 ± 17.2 Hz, wash in: 186.7 ± 16.3 Hz, washout: 189.7 ± 21.8 Hz). Since ACh may be degraded by the acetylcholine esterase (ACh-E), we then coapplied the ACh-E blocker physostigmine (2 μM). This resulted in similar changes of SPW-Rs properties compared with those resulting from 100 μM nicotine application (Supplemental Fig. S2, A and B). In these experiments we found a significant and reversible increase in the SPW-Rs amplitude from 2.8 ± 0.3 to 4.2 ± 0.2 mV (n = 5, P > 0.005, washout: 3.1 ± 0.2 mV) and incidence from 8.2 ± 1.0 to 13.1 ± 1.7 SPW-Rs/min (washout 6.1 ± 0.7), whereas duration and frequency were not significantly altered, similar to the experiments with 100 μM nicotine (for details see Supplemental Fig. S2).

Effects of 100 μM nicotine are α7-nACh receptor-dependent

To investigate in more detail the profile of the receptor subtypes involved in the observed nicotine-mediated effects, we performed experiments with two different, more specific, nACh receptor antagonists. We applied the nonspecific antagonist mecamylamine (MEC, 25 μM) (Giocomo and Hasselmo 2005) to block the two main types of nicotine receptors in the hippocampus, namely α7 and α4-β2 subunit containing receptors (Nott and Levin 2006). In these experiments, MEC was added to aCSF for ≥20 min before 100 μM of nicotine was coapplied during established SPW-R activity. MEC prevented the nicotine-mediated effects on the incidence, amplitude, and duration of SPW-Rs as well as on ripple frequency (n = 6 slices, P > 0.05, each; Supplemental Fig. S3, A and B). Importantly, application of methyllycaconitine (MLA, 10 nM), a specific antagonist for the α7 subunit containing nicotinic receptors, which are strongly expressed in hippocampal interneurons (Séguela et al. 1993), also blocked effects caused by 100 μM nicotine (n = 5 slices; for details see Supplemental Fig. S3). Importantly, the application of MLA did not generate changes in the control activity (Supplemental Fig. S3). However, effects on SPW-Rs mediated by 500 μM of nicotine could not be fully antagonized by both nAChR antagonists, in part due to unspecific effects of the nAChR antagonist (data not shown).

Nicotine dose-dependently modulates single-cell responses during SPW-Rs

To study how nicotine affected the underlying cellular behavior during SPW-R activity, we performed simultaneous extra- and intracellular recordings from CA3 pyramidal cells (see Fig. 3). Under control conditions, 7 of 11 cells displayed compound excitatory postsynaptic potentials (EPSPs) superimposed by one to two APs. We found that nicotine dose-dependently increased the number of APs generated during SPW-Rs (Fig. 3, Aa and Ab). Thus the number of APs was increased from 1.6 ± 0.4 APs/SPW-R under control to 3.2 ± 0.4 APs/SPW-R during application of 100 μM of nicotine (P < 0.001) and to 19.8 ± 2.6 APs/SPW-R on 500 μM nicotine (n = 7 cells, P < 0.001, Fig. 3, Aa and Ab). Moreover, we found that the amplitude of SPW-R-associated compound EPSPs was almost unchanged during 100 μM nicotine (14.1 ± 1.3 mV under control vs. 15.0 ± 0.9 mV during 100 μM nicotine, P > 0.05), whereas 500 μM nicotine caused a significant increase to 20.2 ± 0.8 mV (P < 0.001; Fig. 3). Notably, the four cells, which displayed IPSPs during SPW-Rs, did not change their behavior in the presence of 100 μM nicotine (n = 4 cells, Fig. 3, Ba and Bb). In contrast, when exposed to 500 μM nicotine, cells that originally generated IPSPs during SPW-Rs switched their response into SPW-R-associated EPSPs, generating 4.3 ± 1.4 APs/SPW-R (P < 0.001, n = 4 cells, Fig. 3, Ba and Bb). However, in those cells there were no paroxysmal depolarization shifts. In two cells that were recorded during washout for ≥50 min, we observed that the transition into SPW-R-associated EPSPs was fully reversible (data not shown). In both concentrations, nicotine did not significantly change the input resistance (Rᵢ) of recorded CA3 pyramidal cells (control: 35.7 ± 3.9 MΩ; 100 μM: 38.7 ± 2.7 MΩ; 500 μM: 38.8 ± 3.2 MΩ; and washout: 34.4 ± 3.8 MΩ; n = 11 cells, P > 0.5, each). Similarly, the membrane potential in stimulated slices was not significantly changed before and after application of nicotine in both concentrations (n = 11 cells, P > 0.05; data not shown).

Nicotine dose-dependently affects spike timing of CA3 pyramidal cells during ripple oscillations

To investigate whether nicotine had any effects on the temporal relation of APs to the network ripples, we also determined the jitter between the AP peaks recorded from single CA3 pyramidal cells and the trough of extracellularly recorded ripples during the course of a given SPW-R (n = 1,376; Fig. 4A). Notably, we observed that the increased cellular firing caused by 100 μM nicotine was accompanied by a significant reduction in the jitter between APs to the corresponding ripple oscillations (Fig. 4B). Thus in the presence of 100 μM nicotine, the jitter of the latency was significantly reduced to 76.0 ± 8.9% of control (n = 358 ripples in 7 cells, P < 0.05; Fig. 4C). In contrast, 500 μM nicotine significantly increased the jitter of the latency to 139.1 ± 10.5% of control (n = 852 ripples in 7 cells, P < 0.05; Fig. 4, B and C).

Nicotine reduces the GABAAergic input onto CA3 principal cells

Based on our observations that during 500 μM nicotine, SR stimulation induced multiple PSs in area CA3 and that GABAAergic disinhibition had previously been shown to cause similar changes in area CA3 (Hablitz 1984), we hypothesized that nicotine might reduce GABAA receptor-mediated inhibition onto CA3 pyramidal cells. To test for nicotine-mediated
effects on inhibition, we performed sharp microelectrode recordings of evoked IPSPs during constant positive and negative current pulses in the absence of glutamatergic transmission due to coapplication of DL-APV (50 μM) and CNQX (25 μM).

In these experiments, the SR and SO in area CA3 were alternatively stimulated during application of 100 and 500 μM nicotine (n = 7 cells; Fig. 5, A–C). IPSPs evoked by SR and SO stimulation did not show significant differences (data not shown). However, in the absence of glutamatergic transmission, six of seven cells showed a depolarization of the resting membrane potential of $-63.2 \pm 0.9$ to $-60.5 \pm 1.5$ mV ($P < 0.02$) and to $-58.9 \pm 1.8$ mV ($P < 0.003$) on 100 and 500 μM nicotine, respectively (Fig. 5A). During nicotine application, the amplitudes of evoked IPSPs evoked at depolarizing and hyperpolarizing membrane potentials were reduced in a dose-dependent manner (Fig. 5A).

The remaining IPSPs were completely abolished by BMI (5 μM; data not shown). These changes were not accompanied by a major shift of the chloride reversal potential ($E_{\text{IPSP}}$) during nicotine application, which was $-74.3 \pm 1.5$ mV (control), $-75.1 \pm 1.6$ mV (100 μM; Fig. 5, A and B), and $-75.7 \pm 1.7$ mV (500 μM, $n = 7$ cells, $P > 0.05$; Fig. 5, C and D). When we calculated the synaptic...

**FIG. 3.** Dose-dependent effects of nicotine on intracellular responses during sharp-wave ripples in CA3 pyramidal cells. **Aa**: overlay of 5 samples of simultaneous extra (top) and intracellular recordings (bottom) showing cellular responses of EPSP-generating CA3 pyramidal cell during SPW-Rs under control condition (left) and during application of 100 μM (middle) and 500 μM nicotine. **Ab**: plotted EPSP amplitude (left) and number of action potential (AP)/SPW-Rs (right) under control (white) and during application of 100 μM (gray) and 500 μM (black) nicotine. **Ba**: analogous to A, overlay of 5 samples of simultaneous extra (top) and intracellular recordings (bottom) of inhibitory postsynaptic potential (IPSP)–generating CA3 pyramidal cells during SPW-Rs (APs are truncated). **Bb**: plotted IPSP amplitude (left) and number of AP/SPW-Rs (right) under control (white) and during application of 100 μM (gray) and 500 μM (black) nicotine. Note that in the presence of 500 μM nicotine, IPSP-generating cells switch their response into suprathreshold EPSPs, which under this condition do not represent paroxysmal depolarization shifts as observed in EPSP-generating cells.

**FIG. 4.** Dose-dependent effects of nicotine on the jitter between the peak of APs generated by CA3 pyramidal cells and the trough during synchronized ripple oscillations. **A**: scheme of an AP generated by a CA3 pyramidal cell and its corresponding field ripple showing latency between the ripple trough and AP peak. **B**: plots showing the occurrence of APs (black streaks) during the time windows of ripple-oscillations in seven cells generating EPSP-associated APs during SPW-R activity in the presence of 100 and 500 μM nicotine. Note that in control conditions all cells already fired in a preferential interval during field ripples (CTL, top trace). During 100 μM nicotine (middle trace) cells generated a significantly higher number of APs. Note that the SD around the averaged latency ($\sigma$) was smaller under these conditions, indicating a decreased jitter. In contrast, application of 500 μM nicotine resulted in a significant augmentation of APs (bottom trace), accompanied by an expansion of the SD around the averaged jitter between the AP peak and the corresponding ripple trough, indicating a loss of spike-timing precision. **C**: normalized SD of the jitter between APs and ripple troughs from 1,528 ripples (from 7 cells). Note the significantly decreased SD, indicating an increased spike-timing precision in the presence of 100 μM nicotine ($P < 0.05$).
conductance underlying the generation of IPSPs (see Methods), we found that this was dose-dependently decreased by nicotine from 31.3 ± 5.3 nS (control) to 21.0 ± 5.6 nS (100 μM) and 7.6 ± 1.7 nS (500 μM, n = 7 cells; Fig. 5). Normalization of the absolute data revealed that nicotine caused a significant reduction in the IPSP conductance to 67.0 ± 17.3% of control (100 μM, P < 0.05; Fig. 5B) and to 24.7 ± 4.5% of control (500 μM, P < 0.001, Fig. 5B).

To test whether nicotine-mediated effects on evoked IPSPs were α7-nACh receptor-specific, we applied 100 and 500 μM nicotine in the presence of MLA (10 nM). These experiments revealed that MLA prevented the effect mediated by 100 μM nicotine (n = 6 slices, P < 0.07), indicating that the observed attenuation of the IPSP was α7-nAChR-dependent (Fig. 5, C and D). However, a significant decrease of the evoked IPSP amplitude could not be fully prevented by MLA when 500 μM nicotine was applied (n = 6 slices, P > 0.001). Accordingly, in the presence of MLA, normalized synaptic conductance was not significantly reduced during application of 100 μM of nicotine (97.5 ± 9.1% of control), whereas this was decreased to 49.3 ± 9.2% of control (n = 6, P < 0.001) when 500 μM nicotine was applied (control: 17.6 ± 4.3 nS, 100 μM: 16.8 ± 4.3 nS, 500 μM: 11.0 ± 3.3 nS, n = 6).

Dose-dependent effects of bicuculline on stimulus-induced SPW-Rs

Based on our finding that partial disinhibition, caused by nicotine, could lead to a transition of stimulus-induced SPW-Rs into REDs, we investigated whether partial disinhibition, induced by increasing concentrations of BMI, similarly affected SPW-Rs in area CA3. To detect the threshold of disinhibition needed to induce spontaneous REDs, we applied BMI to naïve slices. Application of 1 μM BMI did not result in the generation of REDs in area CA3 (n = 11 slices; data not shown). When BMI was applied in a concentration of 2 μM, REDs occurred in 54.0% of recorded slices (n = 13 slices), with an incidence of 8.4 ± 1.2 events/min and an amplitude of 5.1 ± 1.1 mV (n = 7, each). Ripple frequency was 237.2 ± 11.6 Hz (n = 7 slices). They lasted on average for 135.3 ± 9.0 ms (n = 7 slices). Notably, in a concentration of 3 μM, BMI reliably induced REDs in all recorded naïve slices. Properties of spontaneous REDs were well comparable to those observed during 2 μM BMI; they occurred with an incidence of 8.4 ± 2.0, showed an amplitude of 6.3 ± 1.5 mV, and lasted for 131.4 ± 7.2 ms, whereas the frequency of superimposed ripples was 241.0 ± 7.2 Hz (n = 5 slices, P > 0.05, each).

Subsequently, we tested for dose-dependent effects of BMI in slices where SPW-Rs had been induced (data not shown) prior to BMI application. When BMI was applied in a concentration of 1 μM, SPW-Rs were transformed into REDs in 36% of 14 slices, whereas in 64% SPW-Rs were increased only in amplitude and ripple frequency (data not shown). In the latter slices, the amplitude of SPW-Rs was enlarged from 3.1 ± 0.2 to 5.0 ± 0.5 mV (P < 0.001) and the frequency of ripples was increased from 190.2 ± 3.2 to 213.0 ± 9.6 Hz (P < 0.02). In contrast, the incidence and duration of SPW-Rs remained unaffected (n = 9, P > 0.09 and P > 0.6, respectively). In the remaining 5 of 14 slices, 1 μM BMI transformed SPW-R into REDs, which lasted for 142.0 ± 7.6 ms (P < 0.001), with an amplitude of 5.5 ± 0.2 mV (n = 5 slices, P < 0.001). Incidence decreased to 7.1 ± 0.3 REDs/min (n = 5 slices). In these slices, the frequency of ripple oscillations was slightly but nonsignificantly increased to 202.9 ± 5.5 Hz (n = 5, P > 0.06). BMI (2 μM) reliably converted SPW-Rs into REDs in all tested slices (data not shown). In these experiments the incidence of REDs was significantly reduced to 7.1 ± 0.7 REDs/min (n = 5 slices, P < 0.004), whereas they showed an amplitude of 7.3 ± 1.3 mV (n = 5 slices, P < 0.02) and lasted for 147.7 ± 3.1 ms (n = 5 slices, P < 0.001; data not shown). As expected, the ripple frequency was significantly increased from 198.2 ± 7.7 Hz during SPW-Rs to 266.3 ± 7.0 Hz (n = 5 slices, P < 0.001). A further increase of BMI concentration to 3 μM showed that properties of REDs were well comparable...
to those recorded during 2 μM BMI (incidence: 7.3 ± 0.8 REDs/min (P < 0.02); amplitude: 8.2 ± 1.3 mV (P < 0.01); duration: 145.8 ± 9.2 ms (P < 0.01); ripple frequency: 262.9 ± 8.4 Hz (P < 0.001)), n = 5 slices, each. P values resulting from comparison to SPW-Rs]. In Fig. 6, the time course of these alterations is depicted. The transformation of SPW-Rs into REDs started 10 min after onset of 2 μM BMI application and was nearly complete 5 min later.

Bicuculline-mediated effects on inhibitory conductance in CA3 pyramidal cells are dose-dependent

To investigate in more detail dose-dependent effects of BMI on inhibitory synaptic transmission onto CA3 pyramidal cells, we next applied BMI at concentrations of 1 and 2 μM on monosynaptic IPSPs evoked in the absence of glutamatergic transmission (see Fig. 7). BMI reduced the amplitude of evoked IPSPs in a dose-dependent manner (see Fig. 7, A and B). Comparison of the inhibitory conductance before and after BMI application revealed that this was reduced, respectively, to 43.3 ± 3.6% of control (1 μM, n = 8 cells, P < 0.001) and to 15.5 ± 4.3% of control (2 μM, n = 6 cells, P < 0.001, Fig. 7C; control: 14.5 ± 1.0 nS, 1 μM: 6.0 ± 0.3 nS, 2 μM: 3.2 ± 1.1 nS). In these experiments input resistance of CA3 cells was not significantly changed (control: 33.2 ± 2.9 MΩ, 1 μM: 34.7 ± 2.5 MΩ, 2 μM: 35.7 ± 2.0 MΩ, P > 0.05, each; see Fig. 7A).

Effects of phenobarbital on induced SPW-Rs

Phenobarbital (PB) enhances IPSPs by increasing mean open time of Cl⁻ channels (Macdonald et al. 1989). To determine whether SPW-Rs are influenced by phenobarbital we investigated the effects of 20 μM PB on established SPW-R activity (Fig. 8). These experiments revealed a significant reduction of amplitudes and incidence of SPW-Rs in area CA3

FIG. 6. Comparison of SPW-R transition into hypersynchronized network activity caused by nicotine (500 μM) or low-dose bicuculline (2 μM). Aa: sample recording showing transition of SPW-Rs into hypersynchronized network discharges during wash in of 500 μM nicotine. Ab: representative CA3 network discharges depicted on enlarge timescale before and during wash in of 500 μM nicotine. Ac: normalized, plotted time courses of duration, incidence, and amplitude of synchronized events and frequency of superimposed network fast oscillations. Bb: analogous to Aa, transition of SPW-Rs into hypersynchronized network discharges following 2 μM bicuculline methiodide (BMI) application. Bb: representative CA3 network discharges shown on enlarge timescale before and during wash in of 2 μM BMI. Bc: normalized, plotted time courses of duration, incidence, and amplitude of synchronized discharges and frequency of superimposed network fast oscillations. Note abrupt occurrence of massively prolonged discharges was observed only during BMI application.
(n = 8, P < 0.05). Notably, the frequency of ripple oscillations superimposed on sharp waves (SWs) was not significantly altered, although a small reversible decrease of the ripple frequency was observed in the presence of PB and subsequent washout (for details see Fig. 8).

**Effects of elevated extracellular potassium concentration on induced SPW-Rs**

The KCC2 transporter exploits the transmembrane potassium gradient to extrude chloride (Cl\(^-\)) from cells (Misgeld et al. 1986; Rivera et al. 1999), thereby setting the reversal potential \(E_{\text{ipsp}}\) to potentials more negative than the resting membrane potential. As previously reported, increasing \([K^+]_o\) shifts the GABA reversal potential in a depolarizing direction (Thompson and Gähwiler 1989) possibly resulting in a reduced efficacy of inhibition (Lopantsev et al. 2009; Staley and Proctor 1999). We therefore tested whether 8.5 mM \([K^+]_o\) converted stimulus-induced SPW-Rs into REDs (see Fig. 9).

Elevation of \([K^+]_o\) from 3 to 8.5 mM resulted in a significant increase of the SPW-R incidence from 10.1 ± 1.6 to 47.8 ± 5.0 events/min (n = 11 slices, \(P < 0.001\); Fig. 9A). Similarly, in the presence of 8.5 mM \([K^+]_o\), the duration of SPW-Rs was prolonged from 45.2 ± 4.7 to 61.6 ± 7.0 ms (n = 11 slices, \(P < 0.001\)), and caused a significant increase in the amplitude from 2.9 ± 0.1 to 6.8 ± 0.8 mV (n = 11 slices, \(P < 0.00\), Fig. 9B). Using ion-sensitive microelectrodes, we found that transient, SPW-R-associated increases in \([K^+]_o\) were augmented from 0.9 ± 0.01 to 0.13 ± 0.02 mM (\(P < 0.05\)) in the presence of 8.5 mM \([K^+]_o\) (Fig. 9C). Interestingly, the frequency of SPW-R-associated ripple oscillation was not significantly altered (180.3 ± 6.8 to 192.6 ± 7.7 Hz, \(n = 11\) slices, \(P > 0.3\); Fig. 9B).

Simultaneous intracellular recordings from CA3 pyramidal cells revealed that 8.5 mM \([K^+]_o\) did not significantly change either the resting membrane potential (62.1 ± 0.4 vs. 60.6 ± 2.8 mV, \(n = 9\) cells, \(P > 0.05\)) or the input resistance (44.8 ± 3.3 vs. 41.0 ± 8.4 MΩ, \(n = 9\) cells, \(P > 0.05\)). In line with previous findings (Staley et al. 1998), in the presence of 8.5 \([K^+]_o\), CA3 cells showed an increased intrinsic excitability, as indicated by a significantly increased firing rate associated with a high incidence of spontaneous burst discharges (see Fig. 9D). In particular, comparison of cellular responses to SPW-Rs in EPSP-generating cells revealed that elevated \([K^+]_o\) caused a significant increase in the number of APs generated during SPW-Rs from 1.6 ± 0.1 to 5.8 ± 0.2 APs/SPW-R (\(n = 5\) cells, \(P < 0.001\); see Fig. 9D). Notably, intracellular recordings from these cells did not reveal prolonged depolarizing responses during network discharges, as observed following application of nicotine and BMI (see Fig. 9D). EPSPs, associated with SPW-Rs, lasted for 51.5 ± 0.1 ms during 3 and 8.5 mM \([K^+]_o\), respectively (\(n = 5\) cells, \(P < 0.01\)). That GABAergic inhibition was preserved under these conditions was indicated by the fact that cells, which displayed IPSPs during SPW-Rs in the presence of 3 mM \([K^+]_o\), also did not generate APs during SPW-Rs when \([K^+]_o\) was elevated (\(n = 4\) cells; see Fig. 9E).
Since SPW-Rs were not transformed into REDs during elevated \([K_+]_o\), we tested for 8.5 mM \([K_+]_o\)-mediated effects on isolated IPSPs in the absence of glutamatergic transmission (see Fig. 10, A and B). Intracellular recordings revealed that in the presence of DL-APV and CNQX the resting membrane potential of CA3 pyramidal cells was significantly depolarized during 8.5 mM \([K_+]_o\) from 64.7 $\pm$ 1.4 to 57.1 $\pm$ 1.7 mV \((n = 7\) cells, \(P < 0.001\)). We found that the \(E_{\text{ISP}}\) was shifted from $-74.7 \pm 1.8$ to $-60.8 \pm 1.3$ mV \((n = 7, P < 0.001, \text{Fig. 10})\). However, in contrast to experiments where both nicotine and BMI caused a significant decrease of the inhibitory conductance in the absence of glutamatergic transmission, we noted a profound increase of inhibitory conductance to 215.2 $\pm$ 38.4% of control during elevated \([K_+]_o\) (from 23.1 $\pm$ 7.2 to 45.8 $\pm$ 12.0 nS, \(n = 7\) cells, \(P < 0.05; \text{Fig. 10, B and C}\)). Moreover, due to the \([K_+]_o\)-induced depolarization of the resting membrane potential, we still observed hyperpolarizing IPSPs, as indicated by the overlay of isolated IPSPs evoked at depolarizing and hyperpolarizing potentials (see Fig. 10A). These recordings also indicated a significant prolongation of evoked IPSPs, in line with previous findings for CA1 neurons (Jensen et al. 1993). The input resistance of CA3 cells was significantly reduced from 41.4 $\pm$ 3.2 to 28.1 $\pm$ 2.6 M$\Omega$ \((n = 7\) cells, \(P < 0.005\)) during elevated \([K_+]_o\).

As previously reported, elevation of \([K_+]_o\), induced spontaneous seizure-like events (SLEs) in different preparations, including human hippocampal tissue (Gabriel et al. 2004; Jandova et al. 2006; Jensen and Yaari 1997; Leschinger et al. 1993). We tested whether under our experimental conditions elevation of \([K_+]_o\), could also induce SLEs. In the presence of 1.2 mM \([Mg_2+]_o\), elevation of \([K_+]_o\) to 8.5 mM caused spontaneous SLEs in 6 of 14 naïve slices (43%; see Fig. 11), whereas this was the only case in one of 12 stimulated slices (8%) expressing SPW-Rs.

**DISCUSSION**

The aim of the present study was to determine the relationship between hypersynchronized REDs and SPW-Rs. We
found that partial disinhibition can convert SPW-Rs into REDs and, interestingly, that SPW-Rs, once induced by a recurrent stimulation protocol reminiscent of a short-term kindling protocol, prevented generation of seizure-like events induced by elevation of \([K^+]_o\). A better understanding of the relationship between SPW-Rs and REDs gained even more importance as spontaneous events, superimposed by ripple network oscillations, have previously been observed in humans and rodents with epilepsy. Thus the question has been posed under what conditions such events indicate the epileptogenic zone in temporal lobe epilepsy. In such regions, inhibition is usually, to some extent, compromised. In the present study, we therefore particularly investigated whether and when partial disinhibition might cause transition of SPW-Rs into interictal, proconvulsant, epileptiform discharges in area CA3 of adult rat hippocampal slices. This study builds on a previous study from our lab (Behrens et al. 2007) in which we showed that high concentrations of bicuculline and of gabazine transformed SPW-Rs into REDs. In that study, we were interested whether disinhibition leads to generation of high-frequency ripples, frequently observed in chronic epileptic tissue. However, although synchronized ripple oscillations could reach frequencies near 400 Hz during the very initial phase of a given RED (Bragin et al. 1999), even complete blockade of GABAA receptors did not cause generation of ultrafast ripples of \(\approx 600\) Hz, as previously observed in epileptic animals and humans (Behrens et al. 2007). In the present study, partial disinhibition led only to a moderate increase in ripple frequencies.

**Properties of SPW-R**

As previously reported, hippocampal SPW-Rs can be induced in vitro by recurrent electrical stimulation used to induce LTP in area CA3 (Behrens et al. 2005), irrespective of stimulation site and irrespective of whether HFS or TBS were used. Induction of SPW-Rs followed induction of LTP and this process was associated with a reorganization within hippocampal networks (Behrens et al. 2005). In a subset of cells, induction of SPW-Rs was associated with augmentation of compound EPSPs, whereas in others inhibition was augmented. Simultaneous intracellular recordings showed that one...
to two APs were observed in about half of the CA3 pyramidal cells, whereas transition into a hypersynchronized network state was prevented by counterbalanced increase of inhibition in others (Behrens et al. 2005). Thus during ongoing SPW-R activity many cells in area CA3 and even more in area CA1 presented with large and long-lasting inhibitory potentials in contrast to epileptiform discharges (Behrens et al. 2005; Maier et al. 2003). In the present study, we confirmed the finding that SPW-Rs propagated into area CA1. In addition to that, we also report a slight propagation of SPW-Rs from area CA3 into the granular cell layer of the DG. This was increased in the absence of GABA<sub>A</sub>-mediated inhibition. However, the observed signals were generally small and might potentially have represented far-field effects or activation of interneurons through recurrent collaterals from CA3 pyramidal cells.

Nicotine in high concentrations transforms SPW-Rs into REDs

Single-pulse stimulation applied to the SR in area CA1 induced network responses in area CA3, which were characterized by two PSs, a first short-latency, antidromic PS followed by a second PS due to the extensive recurrent axon collateral network in area CA3 (Wittner et al. 2007). Although 100 μM of nicotine caused an increase in the amplitude of the second PS, application of 500 μM resulted in the generation of multiple PSs, suggesting removal of recurrent inhibition.

Application of 100 μM of nicotine caused a significant increase in the amplitude of SPW-Rs. Interestingly, this effect of nicotine was sensitive to the α<sub>7</sub>-nAChR antagonist MLA, a specific antagonist of α<sub>7</sub>-nAChRs (Alkondon et al. 1992). In the hippocampus, α<sub>7</sub>-nAChRs are highly expressed in cholecystokinin (CCK)-expressing GABAergic basket cells (Frazier et al. 1998; Freedman et al. 1993). Paired recordings of synaptically coupled interneurons recently showed that activation of CCK-positive basket cells was indeed able to interrupt AP firing in PV-positive interneurons (Karson et al. 2009), thereby causing a reduced inhibitory transmission onto pyramidal cells. Together with this, the present data suggest that nicotine-mediated activation of CCK-positive interneurons might have caused a partial disinhibition of CA3 pyramidal cells. This effect was dramatically increased when nicotine was applied at a dose of 500 μM. Under this condition the amplitude of stimulus-induced SPW-Rs and the duration of SPWs were increased. Cells that presented with compound EPSPs were increased. Cells that presented with compound EPSPs were increased. Cells that presented with compound EPSPs were increased. Cells that presented with compound EPSPs were increased. Cells that presented with compound EPSPs. Application of 100 μM nicotine was not fully sensitive to 7-nAChR (Alkondon et al. 1992). This suggests that nicotine might facilitate induction of SPW-Rs via modulation of CCK-positive GABAergic basket cells that, indeed, express high levels of α<sub>7</sub>-nAChr (Frazier et al. 1998; Freedman et al. 1993). These cells innervate fast spiking parvalbumin-positive basket cells and pyramidal cells (Freund 2003) and might, by disinhibition, facilitate induction of LTP.

Nicotine modulates the GABA<sub>A</sub>-mediated inhibition onto CA3 pyramidal cells

Experiments on isolated IPSPs in the absence of glutamatergic transmission revealed that nicotine indeed reduced IPSPs in CA3 pyramidal cells. The 100 μM nicotine-mediated reduction of inhibitory conductance was α<sub>7</sub>-nAChR-dependent. Nicotine, used in similar concentrations as applied here, showed an α<sub>7</sub>-nAChR-dependent reduction of the GABAergic inhibition in area CA1, where this effect was suggested to contribute to a facilitated induction of LTP in the Schaffer collateral pathway (Zhang and Berg 2007). However, reduction of inhibition mediated by 500 μM of nicotine was not fully sensitive to MLA in the chosen concentration. This may relate to a concentration of the antagonist, which is potentially too low under conditions when 500 μM nicotine is applied. However, MLA developed rather unspecific effects not causally related to nAChR activation when applied in high concentration (data not shown). The observed reduction in inhibition might be due to reduced GABA release resulting from depolarization blockade of interneurons (Alkondon et al. 2000), but might also involve effects on the presynaptic GABA release (Radcliffe et al. 1999) and increased inhibition of soma-inhibiting GABAergic neurons by CCK-positive basket cells, which inhibit both the soma of pyramidal cells (for review see Freund 2003) and other
interneurons (Karson et al. 2009). It is noteworthy that the majority of nicotine-mediated effects observed in the present study are of toxicological interest only because concentrations of 100 and 500 μM nicotine are presumably never reached during consumption of cigarettes. Interestingly, however, 10 μM of ACh has already demonstrated effects similar to those of 100 μM nicotine when ACh esterase activity was reduced by physostigmine coapplication. These effects could be ascribed to activation of nicotinergic receptors because the effects persisted in the presence of the muscarinic receptor antagonist atropine.

Comparison of nicotine- and BMI-mediated effects on the transition of SPW-Rs into REDs

Although 500 μM of nicotine did not induce spontaneous REDs in naïve slices, 3 μM of BMI reliably induced such events. Also 2 μM of BMI induced REDs in a relatively large proportion of naïve slices. At this concentration BMI reliably transformed SPW-Rs into REDs accompanied by increases in [K⁺], of about 1.8 mM. As shown by the present results of isolated IPSPs recorded in CA3 pyramidal cells, this compared with a reduction of the inhibitory conductance by 85%, which was more than the 76% reduction required for the nicotine-mediated transformation of SPW-Rs into REDs. We suggest that this might in part be due to additional effects of BMI. This GABA<sub>B</sub> receptor antagonist not only affects phasic inhibition but also blocks tonic inhibition (Bai et al. 2001). Reduced tonic inhibition has been shown to be involved in the induction of REDs in area CA3 (Glykys and Mody 2006). Moreover, BMI has previously been shown to affect glycnergic inhibition (Shirasaki et al. 1991) as well as SK channels (Debarbieux et al. 1998; Johnson and Seutin 1997; Stocker et al. 1999). Whether these effects are sufficient to explain the differences between nicotine and BMI-mediated effects observed here needs further investigation. Notably, comparison of the kinetics of transformation from SPW-Rs into hypersynchronized network discharges revealed that both BMI and 500 μM nicotine exerted similar effects on the transition of CA3 network activity. Under both conditions, an initial slow increase in the SPW-R amplitude was followed by a rather sudden switch into prolonged network discharges.

Nicotine-mediated effects on the jitter between ripple oscillations and action potentials generated in CA3 pyramidal cells

We found that nicotine, applied after induction of SPW-Rs, reversibly increased the amplitude and number of ripple oscillations during SPW-Rs, with rather mild effects on the amplitude of the underlying SPWs. In the presence of 100 μM nicotine this effect was in part due to an increase in the number of APs from 1.6 ± 0.3 to 3.1 ± 0.7 per SPW-R. On the other hand, we observed a significant change in the jitter of CA3 cells to a given ripple trough during the augmented ripple oscillations. This finding was based on our observation that during 100 μM nicotine the distribution of the latencies between APs and their corresponding extracellular ripple troughs became significantly narrower, as indicated by the reduction of the SD of their distribution (see Fig. 4). In contrast, the latency distribution became wider and the SD increased when 500 μM nicotine was applied. Thereby, the increase in ripple amplitude as observed under these conditions could be ascribed to a significantly increased AP firing rate during a given SPW-R up to about 15.8 ± 2.2 APs/SPW-R. We suggest that an increase in the reliability of spike timing, indicated by changes in the jitter, and the moderate increased number of APs both contribute to the increase in ripple amplitudes, observed in the presence of 100 μM nicotine. In particular, changes observed under conditions of 500 μM nicotine were also related to an increase in the number of ripples and APs but, nevertheless, the observed jitter was significantly increased. This compares to a previous study where we studied the effects of high concentrations of BMI and GABAzine, which showed that spike timing to ripple troughs was relatively imprecise presumably due to back and forward propagation within the recurrent excitationary CA3 network. Our findings also suggest that ripple frequency is not primarily determined by interactions between excitatory and inhibitory cells, as is the case in generation of theta and gamma oscillations in the hippocampus. In fact, augmenting inhibition by barbiturate or by potassium did not result in major changes in the ripple frequency and only when inhibition was completely blocked did ripple frequencies increase to nearly 300 Hz. Thus the ripple frequency seems to be determined by the interaction between excitatory cells in area CA3.

Effects of elevated [K⁺]<sub>i</sub> in naïve and stimulated slices

When [K⁺]<sub>i</sub> was elevated to 8.5 mM the incidence of SPW-Rs was strongly increased possibly due to the fact that all recorded CA3 pyramidal cells were converted into burster cells, which presented with three to five action potentials superimposed on a depolarizing envelope. In the presence of 3 mM [K⁺]<sub>i</sub>, bursting in CA3 pyramidal cells is relatively rare but already increased when recordings are performed in 5 mM [K⁺]<sub>i</sub> (see Miles and Wong 1983). Interestingly, increasing [K⁺]<sub>i</sub> to 8.5 mM resulted in the induction of SLEs in 43% of naïve slices but only in 8% of stimulated slices expressing SPW-Rs. As previously shown, during the stimulus induction of SPW-Rs, repeated application of HFS caused an increase in the amplitude of IPSPs in CA3 pyramidal cells that were inhibited during SPW-Rs (Behrens et al. 2005), indicating an activity-dependent increase in inhibition onto CA3 pyramidal cells. Together with the present finding that the propensity of elevated [K⁺]<sub>i</sub> to induce SLEs was markedly higher in naïve slices than in stimulated slices that expressed SPW-Rs, our data suggest that activity-dependent augmentation of inhibition can prevent transition into SLEs. Since the generation of SPW-Rs is presumably due to mutual interaction among synchronically coupled neurons in area CA3 (Buzsáki et al. 1983), increase in excitatory synaptic input during burst discharges will augment synaptic drive for synchronous depolarization via the extensive recurrent axon collateral network in area CA3 (Wittner et al. 2007). The increase in the amplitude of SPW-Rs observed during elevated [K⁺]<sub>i</sub>, might also be related to an increase in excitability of neurons, as suggested by the increased baseline firing rate noted in all recorded neurons (see Fig. 9). In the presence of 8.5 mM [K⁺]<sub>i</sub>, the frequency of ripple oscillations was not increased. Notably, increases in [K⁺]<sub>i</sub>, induced by single SPW-Rs during elevated [K⁺]<sub>i</sub>, were much smaller than those observed during BMI- and 500 μM
nicotine-induced REDs, suggesting that inhibition was still preserved. This was also suggested by our finding that in cells that were inhibited during SPW-Rs, AP generation was still prevented when $[K^+]_o$ was elevated to 8.5 mM (see Fig. 9). As previously reported, increasing $[K^+]_o$ shifts the GABA reversal potential in a depolarizing direction (Thompson and Gähwiler 1989), possibly resulting in a reduced efficacy of inhibition (Staley and Proctor 1999) due to effects on the KC2 transporter, which exploits the transmembrane potassium gradient to extrude chloride ($Cl^-$) from cells (Misgeld et al. 1986; Rivera et al. 1999). Notably, in the present study, we found that under conditions of elevated $[K^+]_o$, the inhibitory conductance was significantly increased, as indicated by effects on evoked IPSPs in the absence of glutamatergic transmission. This finding suggests that the 8.5 mM $[K^+]_o$-mediated increase in the inhibitory transmission prevented a transition of SPW-Rs into REDs. However, we found that elevating $[K^+]_o$ to 8.5 mM could result in the generation of SLEs in a subset of slices, although inhibition was increased. This implies that other mechanisms might contribute to the generation of ictiform activity, potentially involving generation of cellular bursting behavior (Jensen et al. 1994; Korn et al. 1987; Rutecki et al. 1985), altered GABA<sub>A</sub>-mediated inhibition (Swartzwelder et al. 1986), depolarization-induced increase in presynaptic glutamate release (Oltedal et al. 2008; Staley et al. 1998), and/or reduced glutamate uptake into depolarized astrocytes (Kimelberg et al. 1995).

**Comparison of SPW-Rs and REDs**

It is noteworthy that SPW-Rs recorded in vivo and in vitro differ in some aspects. SPW-Rs induced by our stimulation protocol are prominent in area CA3 from where they propagate through area CA1 to the subiculum (Behrens et al. 2005). In contrast, sharp wave–associated ripple oscillations in vivo are less pronounced in area CA3 (Csicsvari et al. 1999). Moreover, in hippocampal slices, repeated stimulation is used to activate a limited neuronal network, which differs from in vivo conditions where a fully preserved network is involved in the generation of SPW-Rs. That most cells are inhibited during SPW-Rs in area CA1 in vivo compares well with experimental in vitro data (Maier et al. 2003). During the induction of SPW-Rs in vitro ≥50% of CA3 pyramidal cell receive strong synaptic inhibition during SPW-Rs. Notably, the majority of these cells showed increased IPSP amplitudes during given SPW-Rs on repeated stimulation, indicating an activity-dependent augmentation of GABAergic transmission (Behrens et al. 2005). This is in stark contrast to network activity during REDs, where inhibition is usually substantially impaired (Behrens et al. 2007; Hablitz 1984; Schwartzkroin and Prince 1978; Traub and Wong 1982), indicating that stimulus-induced SPW-Rs do not represent classical interictal discharges. Indeed, REDs prominently differ from SPW-Rs by a prolonged duration of hypersynchronized network discharges, the development of paroxysmal depolarization shifts accompanied with high-frequency AP firing during epochs of fast ripple oscillations >250 Hz, and prominent increases in $[K^+]_o$ (Behrens et al. 2007) as well as by a significantly increased oxygen consumption detected within the CA3 pyramidal layer during REDs (unpublished data). Interestingly, ripple oscillations in vivo have also been observed in epileptic humans and rodents (Bragin et al. 1999a,b; Staba et al. 2004). In epileptic patients high-frequency oscillations (HFOs) were reported presenting either with ripple frequencies of 80–250 Hz, similar to those recorded during SPW-Rs in our experiments, or fast ripple frequencies (250–500 Hz) (Le Van et al. 2008; Staba et al. 2002). A recent study investigated whether the incidence of HFOs occurring with ripple frequencies might specifically indicate the site of epileptogenesis (Jacobs et al. 2008), which was not the case. Indeed, network events, involving synchronized inhibitory activity, seem to slow spread of SLEs (Trevelyan et al. 2007) and interfere with ictogenesis (Barbarosie et al. 2002). However, since SPW-Rs are usually associated with robust network inhibition, they might represent an intrinsic, dynamic mechanism to modulate the threshold for seizure induction.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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