Pergamon

Neuropharmacology 43 (2002) 573–583

NEURO PHARMACOLOGY

www.elsevier.com/locate/neuropharm

Release-independent short-term facilitation at GABAergic synapses in the olfactory bulb

Z. Nusser *

Laboratory of Cellular Neurophysiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Szigony Street 43, 1083 Budapest, Hungary

Received 10 May 2002; received in revised form 12 July 2002; accepted 17 July 2002

Abstract

Neurones of the olfactory bulb are innervated by GABA-releasing axons and dendrites of diverse origin. Here, I studied GABAergic neurotransmission in juxtaglomerular cells using whole-cell voltage-clamp recordings in acute olfactory bulb slices. Spontaneous IPSCs were fully blocked by the $GABA_A$ receptor antagonist SR95531 (40 μM) and the sodium channel blocker tetrodotoxin (1 μM). The IPSCs had mean amplitudes of 125 ± 86 pA and relatively slow biexponential decay times (τ_1 = 4.3 ± 1.0 ms (67 ± 12%), τ_2 = 16.9 ± 2.7 ms) at physiological temperatures. Short-term plasticity of evoked IPSCs showed two distinct patterns: depressing (n = 4 cells) and facilitating-depressing (n = 9). In two cells, postsynaptic responses were mediated by single functional release sites. During a train of stimuli (4 stimuli at 20 Hz), the release probability increased by two-fold, whereas the potency (postsynaptic responses excluding failures) decreased by ~15%. The increase in release probability for the second stimulus in the train also occurred when the first action potential failed to release transmitter. However, the decrease in the potency was only observed if the preceding action potential released transmitter. These results reveal a heterogeneity in the short-term plasticity of evoked IPSCs in juxtaglomerular cells and demonstrate that the short-term facilitation at some GABAergic synapses is independent of release.

Keywords: GABAA receptors; Patch-clamp recording; Short-term plasticity; Periglomerular cell; Inhibition; Desensitisation

1. Introduction

Synchronous neuronal activity is believed to be essential for many CNS functions, such as neuronal development, memory formation, and sensory perception (reviewed by Buzsaki et al., 1994; Buzsaki and Chrobak, 1995; Laurent, 1999; Singer, 1999). In several brain areas, including the mammalian main olfactory bulb, $GABA_A$ receptor-mediated inhibition is essential for the generation of neuronal population synchrony, which is often manifested as local field potential oscillations (reviewed by Buzsaki and Chrobak, 1995; Singer, 1996; Traub et al., 1998). Understanding the mechanisms of oscillatory synchronization requires a knowledge of the intrinsic properties of the participating cells, their connectivity patterns, and the precise behaviour of synaptic connections. In the olfactory bulb, several of these parameters have been investigated in detail using molecular, morphological and functional approaches. For example, GABAergic synaptic neurotransmission has been extensively studied in mitral/tufted cells (the projecting neurons; Nicoll, 1971; Jahr and Nicoll, 1982; Isaacson and Strowbridge, 1998; Schoppa et al., 1998; Chen et al., 2000) and granule cells (GABAergic local interneurons; Nusser et al., 1999). However, little is known about the kinetics, pharmacological properties, or the short-term plasticity of inhibitory postsynaptic currents (IPSCs) in juxtaglomerular cells. The majority of the latter are presumed GABAergic periglomerular cells, but they also include some glutamatergic external tufted cells and some tyrosine hydroxylase positive cells (Shepherd and Greer, 1990). Periglomerular cells can also be divided according to their calcium binding protein content (e.g. calbindin, parvalbumin, calretinin; Kosaka et al., 1998), but the functional significance of this categorization remains unclear. The major aim of the present study was

* Tel.: +36-1-210-9983; fax: +36-1-210-9984.
E-mail address: nusser@koki.hu (Z. Nusser).

0028-3908/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0028-3908(02)00158-2

to characterize the kinetic properties and the short-term plasticity of $GABA_A$ receptor-mediated postsynaptic responses in juxtaglomerular cells using whole-cell voltage-clamp recordings in vitro.

2. Methods

2.1. Acute slice preparation and whole-cell recordings

Seventeen to twenty two-days old (18.7 ± 1.4 (mean ± SD), $n = 11$) Wistar rats were anaesthetized first with halothane and then with ketamine (50 mg) in accordance with the guidelines of the Institute of Experimental Medicine Protection of Research Subjects. After decapitation, the brains were removed and were placed into ice-cold artificial cerebrospinal fluid (ACSF). Horizontal slices of the olfactory bulb, 350 μm thick, were cut with a Vibratome (Leica VT1000S; Leica Microsystems, Vienna, Austria) and were stored in continuously oxygenated ACSF (pH = 7.4), containing (in mM) 85 NaCl, 75 sucrose, 2.5 KCl, 25 glucose, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 4 $MgCl_2$, and 0.5 $CaCl_2$. After 30 min this medium was replaced by an ACSF, containing (in mM) 126 NaCl, 2.5 KCl, 25 glucose, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 2 $MgCl_2$, and 2 $CaCl_2$. After another hour of incubation at 30°C, the slices were transferred to a recording chamber where they were perfused with ACSF, containing the ionotropic glutamate receptor antagonist kynurenic acid (3 mM). Recordings were performed at 24–36°C from the somata of visually identified cells (Olympus BX51WI microscope with infrared differential interference contrast optics with a 40× water immersion objective) with cell bodies located at the periphery of the glomeruli. Recordings were made with an intracellular solution, containing (in mM) 135 CsCl, 4 NaCl, 1 $MgCl_2$, 0.05 EGTA, 10 HEPES, 2 Mg-ATP, 0.4 Mg-GTP, 10 creatinine-phosphate, 5 QX314, and 0.013 biocytin. The intracellular solution was titrated to a pH of 7.2 and an osmolarity of 290 mosmol. Inhibitory postsynaptic currents were evoked by extracellular stimulation (stimulus isolator made by Supertech, Pécs, Hungary). The stimuli (200 μs width) were delivered through a theta glass pipette filled with ACSF. The synaptic currents were recorded with a Multiclamp 700A amplifier (Axon Instruments, Foster City, CA). Patch electrodes were pulled with a Zeitz Universal Puller (Zeitz-Instrumente Vertriebs GmbH, Munchen, Germany) from thick-walled borosilicate glass capillaries (o.d. = 1.5 mm, i.d. = 0.86 mm, Sutter Instr. Co., Novato, CA). The DC resistance of the electrodes was 3-5 MΩ when filled with pipette solution. Series resistance and whole-cell capacitance were estimated by compensating for the fast current transients evoked at the onset and offset of 10 ms 5 mV voltage-command steps and were checked every two minutes during the recordings. A series resistance (Rs) compensation of 70–90% was applied. The uncompensated Rs was 10.4 ± 5.2 MΩ.

2.2. Chemicals and drugs

Tetrodotoxin was purchased from Calbiochem (La Jolla, CA, USA). Kynurenic acid, SR95531, zolpidem, biocytin and all other chemicals for the intra- and extracellular solutions were obtained from Sigma.

2.3. Data analysis

All recordings were low-pass filtered at 3 kHz and digitised on-line at 20 kHz using a PCI-MIO 16E-4 data acquisition board (National Instruments, Austin, TX) and an in-house data acquisition software (Stimulog1.1, written in LabView). Spontaneous IPSCs were detected and analysed with in-house analysis software (EVAN1.3, written in LabView; see Nusser et al., 2001). The amplitudes, 10–90% rise times, 67% decay times, inter-event intervals were measured in EVAN1.3 and the values were exported to Origin6.1 (OriginLab Corp. Northampton, MA) for further analysis and plotting. The decay of the averaged currents was fitted with either a single or the sum of two exponential functions and the goodness of fit was evaluated with the F test (significance was at $p < 0.01$). The weighted decay time from the fit was calculated as:

$$\tau_{w(f)} = \tau_1 * A_1 + \tau_2 * (1 - A_1)$$

where τ_1 and τ_2 are the time constants of the first and second exponentials, and A_1 is the amplitude contribution of the first exponential. Data are given as mean ± SD, unless otherwise stated.

2.4. Modelling the short-term plasticity

The short-term plasticity of evoked IPSCs (eIPSCs) was investigated using a train of 4–6 stimuli at 20 Hz, followed by a single recovery pulse at 300 ms. This protocol was repeated every 20 s. The amplitude of the averaged eIPSCs was measured in each cell. Two patterns of short-term plasticity were observed: depressing and facilitating-depressing. The amplitudes of the eIPSCs were normalised to that of the first eIPSC in the train and the amplitude values were averaged within each short-term plasticity category and the mean ± SD were plotted (Fig. 3). The discrete version of the dynamic neurotransmitter model developed by Markram and his colleagues (Tsodyks and Markram, 1997; Markram et al., 1998) was implemented in the differential equation solving software *Berkeley Madonna* (R.I. Macey & G.F. Oster, UC Berkeley, CA). The model was fitted to the mean data points, and the parameters of τ_{rec} (time constant of recovery of synaptic efficacy), τ_{fac} (time constant

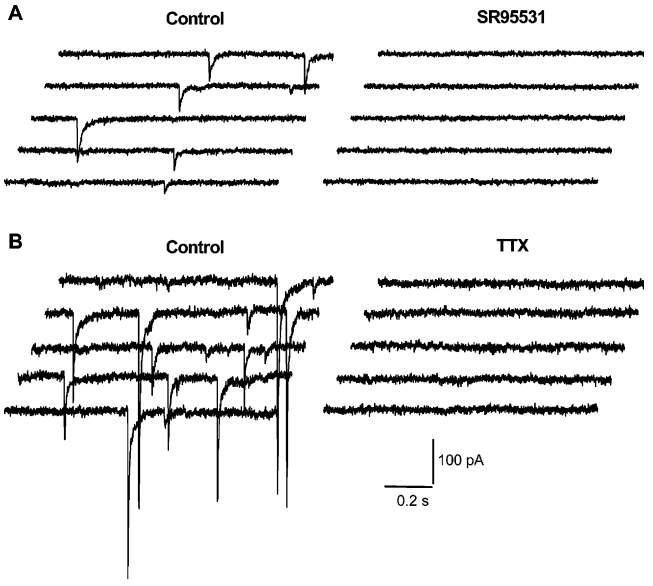

Fig. 1. Spontaneous IPSCs are mediated by $GABA_A$ receptors. (A) Five seconds of continuous whole-cell voltage-clamp records are shown prior to (left) and following (right) the application of the competitive $GABA_A$ receptor antagonist SR95531 (40 μM). (B) All spontaneous IPSCs disappear following the application of 1 μM tetrodotoxin, indicating that these events are action potential-dependent.

of recovery from facilitation), *A* (absolute synaptic efficacy), and *U* (utilized synaptic efficacy) were determined.

2.5. Identifying single release sites

Interpretation of parts of this study rely on the identification of synaptic connections as being mediated by single release sites. The criteria for this identification is described below. To establish that a connection is mediated only by a single functional release site, the connection should be studied under different release probability (P_r) conditions (Silver et al., 1996). This can be achieved by changing $[Ca^{2+}]_e$ (Katz and Miledi, 1968), or by applying trains of stimuli at various frequencies to produce different release probabilities at each stimulus of the train, due to the short-term plasticity of the synapses. In order to reliably identify changes in P_r, there should be a significant number of failures and an obvious change in the failure rate during the train of stimuli. In three out of fifteen cells recorded here, there were several response failures for the first stimuli, and the probability of failure (P_f) changed during the train as expected from changes of the averaged postsynaptic responses (in two facilitating cells P_f decreased and in one depressing cell P_f increased). In one of these cells, the amplitude of the eIPSCs varied as much as three-fold and the potency changed during the train, as expected if multiple release sites mediated the events. In the remaining two cells, the initial failure rates were large (0.68 and 0.79, see Figs. 5B and 6F), and there was a large decrease in the P_f during the train (from 0.68 to 0.38, from 0.79 to 0.47). However, the potency of the responses did not show a corresponding increase as expected at connections mediated by multiple release sites. Using a simple binomial model, I have calculated the expected increase in potency from the 1st to the 4th (3rd) stimulus assuming that two release sites mediated the connection (see equation 5 in Silver et al., 1996). The calculations indicated that, in approximately 15% of the cases both release sites should have released transmitter at the end of the train. This should have resulted in a 12% and a 13% increase in potency, for the two sites respectively. Instead, an 18% decrease in potency from the 1st to the 4th stimulus for the cell illustrated in Figs. 4 and 5, and a 14% decrease in potency from the 1st to the 3rd stimulus for the cell illustrated in Fig. 6 were observed. These results are inconsistent with multiple release sites mediating the evoked responses in these two cells. If the responses were mediated by multiple release sites with drastically different release probabilities, the calculations would not hold true (see Fig. 5D in Silver et al., 1996). If this is the case, the P_r should be very small at one of the release sites, which would rarely contribute to the postsynaptic response, making the connection appear to have only a single release site. Furthermore, a very robust desensitisation of the postsynaptic receptors could, at least in theory, sufficiently reduce the potency to completely mask the expected increase in potency due to the enhanced P_r at multiple site connections.

3. Results

3.1. Characterization of the spontaneous $GABA_A$ receptor-mediated IPSCs in juxtaglomerular cells

Whole-cell voltage-clamp recordings were obtained from the somata of visually identified juxtaglomerular cells in acute slices of the main olfactory bulb of P19 rats. Because symmetrical Cl^- concentrations were used and the cells were held at −70 mV, $GABA_A$ receptor-mediated IPSCs were inward. Spontaneous synaptic currents occurred apparently randomly with frequencies ranging from 1 to 10 Hz (Figs. 1 and 2D). Following the application of the $GABA_A$ receptor antagonist SR95531 (40 μM) all spontaneous inward currents were blocked, demonstrating that all sIPSCs were mediated by $GABA_A$ receptors (Fig. 1A). In all tested cells ($n = 3$), the application of the sodium channel blocker tetrodotoxin (TTX) resulted in a complete disappearance of sIPSCs (Fig. 1B), demonstrating that the sIPSCs were action potential-dependent. As no miniature IPSCs could be isolated in these cells, kinetic analysis was performed on sIPSCs.

The amplitudes of sIPSCs showed large variability in

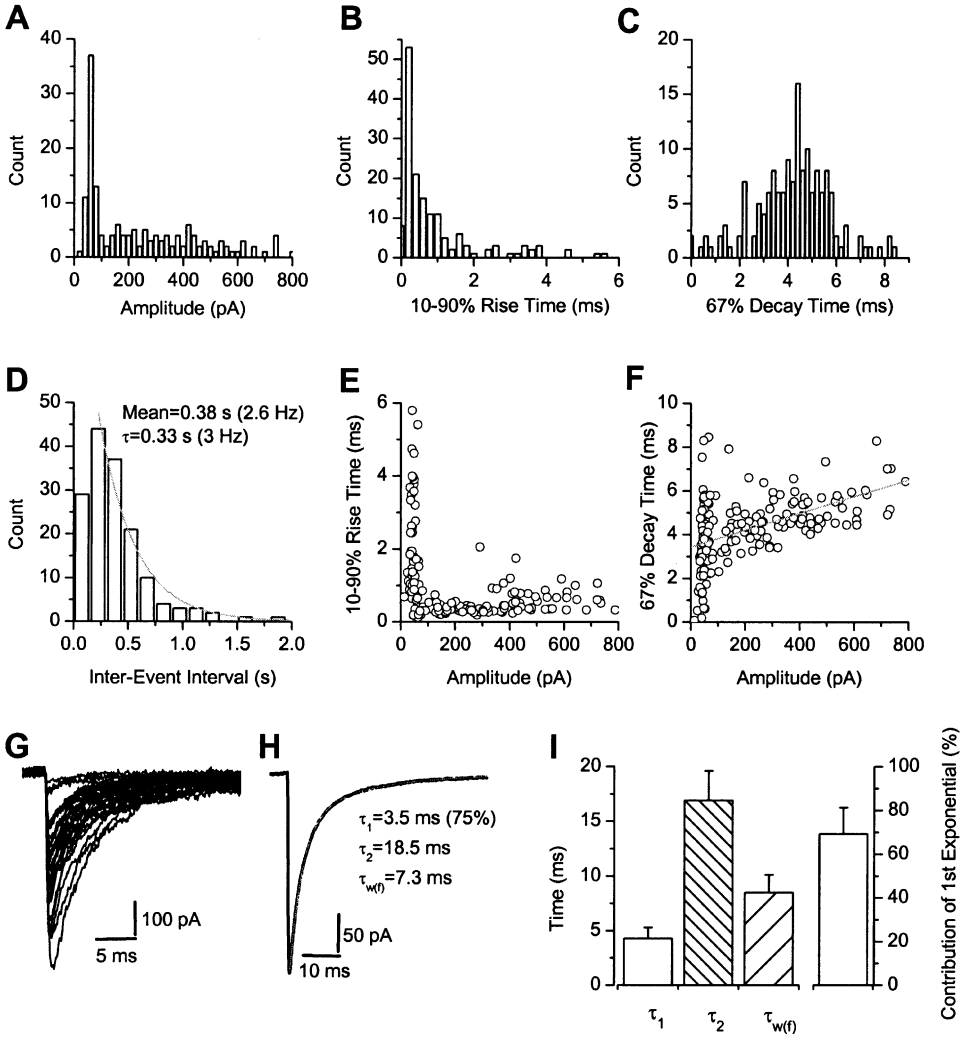

Fig. 2. Characterization of sIPSCs in a juxtaglomerular cell. (A) The distribution of the sIPSC amplitudes is positively skewed with a mean of 229 pA and a SD of 202 pA. (B) The distribution of the 10–90% rise times is positively skewed with a large proportion of the events having rise times less than 400 μs, but others have rise times up to 6 ms. (C) The distribution of the 67% decay times showed relatively small variance (coefficient of variation = 37%). Note that for small IPSCs, the 67% decay time values are substantially reduced by the recording noise compared to that obtained following an exponential fitting. (D) An exponential fit to the distribution of the inter-event intervals yielded an average frequency (3 Hz) very similar to that obtained from the mean of the inter-event intervals (2.6 Hz), indicating the approximately random occurrence of the events. (E) Amplitude versus 10–90% rise time plot. Note that the slowly rising events all have small amplitudes, but the fast rising events could be of any size. (F) There is a significant positive correlation between the amplitude and the 67% decay times of the events (linear regression line in gray, $R = 0.49$, $p < 0.0001$). This is inconsistent with dendritic filtering being solely responsible for the amplitude variability. (G) Twenty-eight consecutive, non-contaminated sIPSCs with 10–90% rise times less than 400 μs are superimposed. Note the large variability in their amplitudes. (H) The average (black trace) of the traces shown in panel G and the superimposed double exponential fit (gray trace) to the decay. (I) The mean ± SD values of the τ_1, τ_2, and $\tau_{w(f)}$ are shown on the left ($n = 7$ cells). The amplitude contribution of the first exponential is plotted on the right.

all cells, resulting in positively skewed amplitude histograms (Fig. 2A). The 10–90% rise times and 67% decay times of the events also displayed a remarkable variability (Fig. 2B and C). The correlation between the amplitudes and rise times (Fig. 2E) pointed to a possible distal dendritic origin of some of the slowly rising IPSCs, which were presumably severely filtered. However, the positive correlation between the peak amplitude and 67% decay time of the sIPSCs (Fig. 2F) is not consistent with dendritic filtering being solely responsible for the variation in amplitudes and decay times. In order to reveal the real amplitude variability and kinetics of unfiltered IPSCs, I selected sIPSCs with 10–90% rise times <400 μs, because such fast rising IPSCs have been found in electrotonically compact cells, where dendritic filtering does not affect the kinetics of the IPSCs (Borst et al., 1994; Bier et al., 1996; Brickley et al., 1996). The amplitude variability also remained remarkably large when only these fast rising sIPSCs were selected (Fig. 2G). One likely source of this variability is the action potential evoked simultaneous release of multiple quanta, but variable quantal sizes at distinct release sites

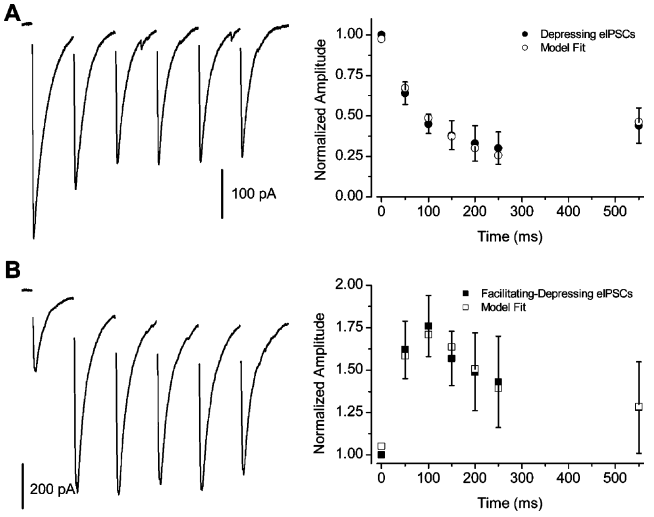

Fig. 3. Short-term plasticity of evoked IPSCs. (A) Averaged IPSCs evoked in a representative cell by 6 stimuli at 20 Hz display reduced amplitudes throughout the whole train. Right panel: Plot of the mean ± SEM values (filled circles) superimposed by the values (open circles) from the fit of the dynamic neurotransmission model. Parameter values are: A = 2.92, U = 0.33, τ_{rec} = 666 ms, τ_{fac} = 3.5 ms. (B) A representative averaged trace showing a facilitating-depressing pattern of short-term plasticity. Right panel: Plot of the mean ± SEM of normalized IPSCs amplitudes evoked by the stimulus train and by the recovery pulse. The result of the best model fit are superimposed (A = 8.3, U = 0.13, τ_{rec} = 201 ms, τ_{fac} = 254 ms). Stimulus artefacts have been digitally removed.

may also contribute to the amplitude variability. The mean ± SD of the 10–90% rise times of the averaged, selected sIPSCs was 0.34 ± 0.06 ms (n = 7 cells) and their amplitudes were 125 ± 86 pA, corresponding to 1.8 ± 1.2 nS peak conductance. The decay of the averaged synaptic currents was always fitted significantly better ($p < 0.01$, F test) by the sum of two exponentials than a single exponential. The mean±SD of the time constants of the 1st and 2nd exponentials were 4.3 ± 1.0 ms and 16.9 ± 2.7 ms, respectively with the 1st exponential contributing 67 ± 10% to the total amplitude (Fig. 2H and I). The weighted decay time was 8.5 ± 1.6 ms at 34.5 ± 1.7°C (n = 7 cells). I also investigated the dependence of the decay time on the recording temperature and found a significant negative correlation with a Q_{10} of 2.0 between 26°C and 36°C.

3.2. Short-term plasticity of evoked IPSCs

It is well known that central synapses display a wide repertoire of dynamic behaviours (e.g. depression, depletion, facilitation and augmentation) on time-scales ranging from tens of milliseconds to several seconds. This behaviour of chemical synapses influences the reliability of the connections upon repetitive activation. The short-term plasticity of GABAergic synapses was tested in juxtaglomerular cells by evoking 4–6 postsynaptic responses at 20 Hz with a recovery test pulse at

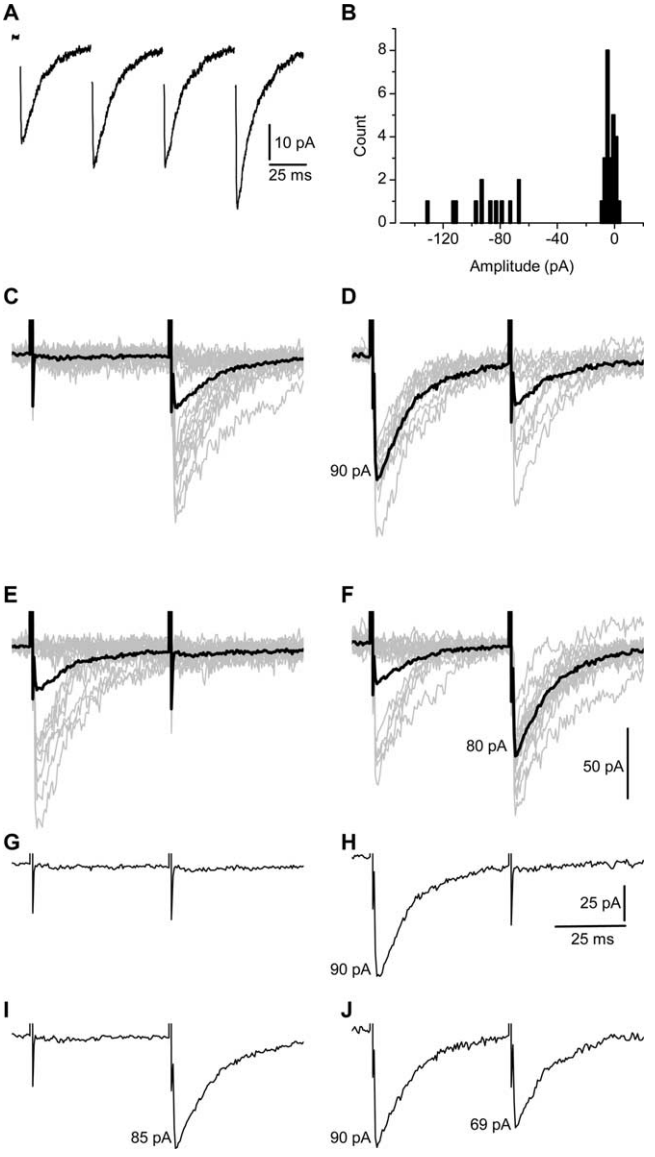

Fig. 4. Evoked IPSCs mediated by a single release site. (A) An averaged current trace shows 4 IPSCs evoked at 20 Hz. The stimulus artefacts have been digitally removed. (B) Amplitude histogram of the IPSCs evoked by the 1st stimuli. The failures and responses can be easily distinguished. The minimum, maximum, mean, SD, and CV values of the successes were 66 pA, 131 pA, 91.2 pA, 19.7 pA, and 0.22, respectively. (C-F) Individual traces are shown in gray and the averaged traces are in black. Traces were grouped according to whether the responses to the 1st stimuli were failures (C) or successes (D) and whether those to the 2nd stimuli were failures (E) or successes (F). The P_r for the 1st stimuli was 0.32 and it increased to 0.43 for the second stimuli. The potency of the 1st responses was 90 pA, whereas that of the 2nd responses was only 80 pA. (G–J) Averaged traces are shown in response to the first two stimuli of the train grouped according to whether failures are followed by failures (G); successes followed by failures (H); failures followed by successes (I); and successes followed by successes (J). The P_rs for the 2nd stimuli were almost identical irrespective of whether the 1st stimuli failed (I, P_r = 0.44) or succeeded (J, P_r = 0.42) to release transmitter. The potency of the 2nd responses depended on whether the 1st stimuli failed (I, 85 pA) or succeeded (J, 69 pA) to release transmitter. Note that the potency of the 1st response does not depend on whether it is followed by a failure (H, 90 pA) or by a response (J, 90 pA).

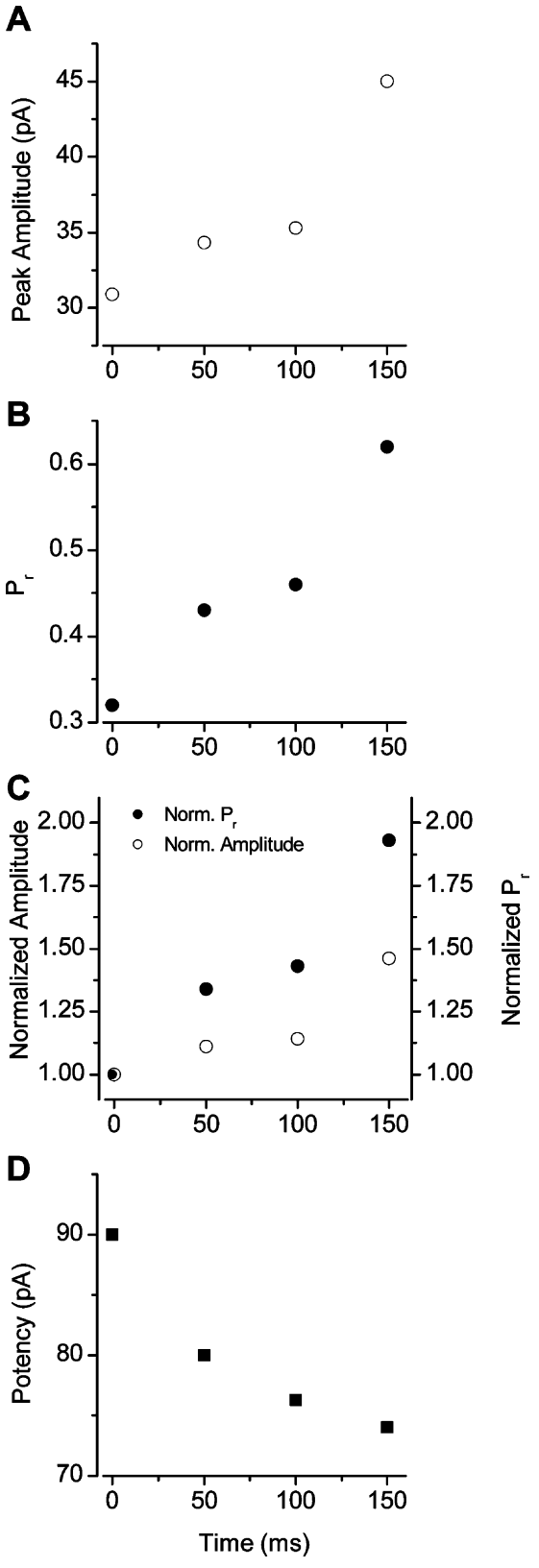

Fig. 5. Summary of the changes in the postsynaptic amplitude (A), release probability (B), and potency (D) during the train of 4 stimuli at 20 Hz. There is a similar pattern of increase of the postsynaptic amplitude (A) and the P_r (B). However, if the normalized changes are compared (C), it is apparent that the increase of the P_r much larger than that of the amplitudes. This is the consequence of a corresponding decrease in potency (D).

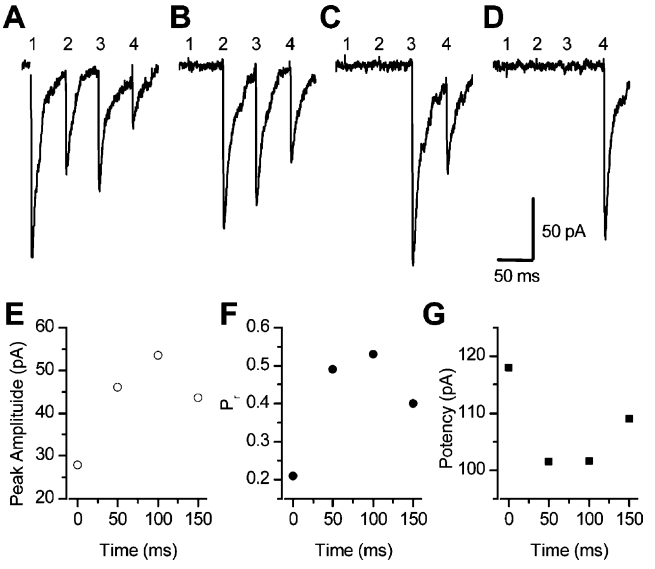

Fig. 6. Release-dependent reduction of the potency at a single site connection. (A–D) Consecutive single traces of evoked IPSCs, selected based on whether all 4 stimuli released transmitter (A), the 1st failed but all others released (B), the first two failed (C), or the first three failed and only the last released (D). Note that the amplitudes of the first IPSCs were very similar, irrespective whether they were the responses to the 1st (A), 2nd (B), 3rd (C), or 4th (D) stimuli. The amplitudes of the 2nd IPSCs were consistently smaller than those of the 1st IPSC. (E and F) During the 4 stimuli, the averaged postsynaptic responses first increase then decrease in amplitudes. A similar pattern is seen for the P_r. (G) The potencies of the responses decrease for the first three stimuli, then increase.

300 ms (Fig. 3). Extracellular stimuli were delivered through theta glass stimulating electrodes with tip diameters of a few μm to minimize the number of fibres activated. GABAergic fibres innervating juxtaglomerular cells are very scarce, as large numbers of stimulation sites had to be tested to find a successful stimulation site, indicating that most of the time probably only a single fibre was stimulated. It is important to note that most (13 out of 15 stimulation sites) fibres made several synapses/release sites onto the postsynaptic recorded cells, as judged from the size, amplitude variability, and the failure rate of the postsynaptic responses (see Methods).

The dynamic behaviour of the postsynaptic responses fell into two distinct categories (Fig. 3). In four cells (Fig. 3A), the amplitude of the averaged responses decreased throughout the whole train of stimuli. The response to the 6th stimulus was ~30% of the 1st response and the postsynaptic response recovered to 44% at 300 ms after the last stimulus. This pattern of short-term plasticity (depression) was well described by the dynamic neurotransmission model developed by Markram and his colleagues (Tsodyks and Markram, 1997; Markram et al., 1998). Model fitting resulted in a very small value of τ_{fac} (3.5 ms), a large value of τ_{rec} (666 ms), and an intermediate value of U (0.33). More

commonly (9 cells), I observed a pattern of short-term plasticity as illustrated in Fig. 3B (facilitating-depressing pattern). This pattern was also well approximated by the dynamic model with very similar τ_{fac} (254 ms) and τ_{rec} (201 ms) values and a small U value (0.13).

3.3. Evoked IPSCs at single release sites

In two out of the fifteen cells where postsynaptic responses were successfully evoked, the postsynaptic responses were mediated by a single release site (for identification see Methods). Both connections showed facilitating-depressing patterns of short-term plasticity. Because in these connections, the number of quanta could be counted for each stimulus, I could determine the change in the P_r during the train of 4 stimuli at 20 Hz. In the cell illustrated in Figs. 4 and 5, the P_r increased from 0.32 to 0.62 from the 1st to the 4th stimuli (Fig. 5B). Next, I investigated whether the increase in P_r depended on the release of transmitter. A P_r of 0.44 was calculated for those 2nd stimuli that followed failures of the first stimuli (Fig. 4I) and 0.42 for those that followed successes of the 1st stimuli (Fig. 4J), indicating that the increase of P_r in this cell was independent of a successful release event. An almost identical result was found in the other single site connection. Namely, the P_r for the first stimulus was 0.21 and it increased to 0.49 for the second stimulus (Fig. 6F). For those events where the 1st stimuli failed to evoke postsynaptic responses, the P_r for the second stimuli (0.48) was almost identical to the P_r for all second stimuli (0.49). The observation in this cell further supports the release-independent increase in P_r during repetitive stimulation at some GABAergic synapses. These experiments also demonstrate that the initial release probability is relatively low (0.21 and 0.32) at those GABAergic synapses that show robust short-term facilitation.

When the increases of the averaged postsynaptic responses were compared to the increases in P_r (cf. Fig. 5A and B), qualitatively similar patterns were observed. However, when the normalized increases in P_r and in average amplitudes were compared (Fig. 5C), the enhancement of the averaged postsynaptic responses was found to be much smaller than that of the P_r. This was the consequence of a significant decrease ($p < 0.05$, unpaired t-test) in the potency of the responses from the 1st to the 4th stimuli (Fig. 5D). To investigate whether the decrease in the potency depended on the release of transmitter, the dependence of the average potency of the 2nd stimulus was investigated on the success (Fig. 4J) or failures (Fig. 4I) of the response to the 1st stimulus. Fig. 4I and J illustrate that the average potency of those 2nd responses that followed successes (69 pA) was smaller than the potency of those 2nd responses that followed failures (85 pA). To analyse the release-dependent decrease in the potency further, consecutive traces were selected from the other connection when the first four stimuli released transmitter (Fig. 6A), the 1st failed, but the rest released (Fig. 6B), the first two failed and the last two released (Fig. 6C), and only the last released transmitter (Fig. 6D). It was apparent that the amplitudes of the 1st IPSCs were very similar irrespective whether they were evoked by the 1st, 2nd, 3rd or 4th stimuli. The decreases in the amplitudes of the 2nd, 3rd and 4th IPSCs were also evident. These results demonstrate that at these synapses the decrease in the potency during the train of 20 Hz stimuli was release-dependent. The number of channels calculated to be open at the peak of the 1st evoked IPSCs was 29 and 37, respectively for these two single site connections (single channel current: 3.2 ± 0.3 pA, $n = 5$ cells; calculated by peak-scaled non-stationary fluctuation analysis of sIPSCs with 10–90% rise times <400 μs). The calculated upper limit of the channel open probability (for calculation see, Silver et al., 1996) was 0.69 and 0.62, resulting in a lower limit of the number of channels at these two synapses of 42 and 59, respectively.

4. Discussion

The present study shows that action potential-dependent $GABA_A$ receptor-mediated IPSCs occur spontaneously in juxtaglomerular cells of the main olfactory bulb of P19 Wistar rats. The relatively slow decay of these currents is consistent with a composition of the underlying $GABA_A$ receptors other than $\alpha_1\beta_x\gamma_2$ (see below). Evoked IPSCs showed two patterns of short-term plasticity; 'depressing' and 'facilitating-depressing'. Some GABAergic inputs to juxtaglomerular cells in vitro are mediated by connections with only single release sites. At these connections, the increase in the release probability during 20 Hz stimulations was independent of vesicular release of transmitter, suggesting that when failure of release occurs during a train it reflects events downstream of action potential invasion and calcium influx. However, the decrease in potency of the postsynaptic responses during the train was release-dependent, pointing to postsynaptic receptor desensitisation as the underlying mechanism.

4.1. $GABA_A$ receptors probably lack the α1 subunit in juxtaglomerular cells

$GABA_A$ receptors are heteromultimeric ion-channels, most of them composed of two α, two β and one γ subunit. Less frequently, the γ subunit is replaced by either a δ, ε, or π subunit (Sieghart, 1995; McKernan and Whiting, 1996; Tretter et al., 1997; Barnard et al., 1998). Several functional properties; including the conductance, agonist affinity, opening probability and desensitisation; are determined by the subunit composition of the recep-

tors (Tia et al., 1996b; Lavoie et al., 1997; Haas and Macdonald, 1999; Bianchi et al., 2001). As gating and desensitisation properties of the postsynaptic receptors are the major determinants of shaping the decay kinetics of the IPSCs (Jones and Westbrook, 1996), the synaptic current decays also depend on the subunit composition. Indeed, very different IPSC decay times were reported in nerve cells expressing $GABA_A$ receptors with distinct subunit compositions. In particular, $GABA_A$ receptors with $\alpha_1\beta_2\gamma_2$ subunit composition are expressed by cerebellar stellate cells (Laurie et al., 1992; Persohn et al., 1992; Nusser et al., 1999) and the weighted decay time of mIPSCs in these cells is 3.2 ± 0.9 ms at 33°C (Nusser et al., 2001). Olfactory bulb granule cells express α2, α5, β3, γ2 and δ subunits (Laurie et al., 1992; Fritschy and Mohler, 1995; Nusser et al., 1999), probably forming $\alpha_2\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$, and $\alpha_x\beta_3\delta$ $GABA_A$ receptors with a weighted decay time of mIPSCs of 15.5 ± 4.4 ms at 33°C (Nusser et al., 1999), which increases to 45 ms at room temperature (Hajos et al., 2000). Very slow IPSC decay times (~100 ms at room temperatures) were also reported in neurones lacking the α1, β2 subunits such as those in the nucleus reticularis thalami (Zhang et al., 1997). Intermediate weighted decay times (3.5–6 ms) were found in dentate gyrus granule cells, in neocortical layer II/III, and hippocampal pyramidal cells at physiological temperatures (Poisbeau et al., 1997; Williams et al., 1998; Nusser et al., 2001). These cells express a wide repertoire of $GABA_A$ receptor subunit, including the α1, β2, and γ2 subunits. In the juxtaglomerular cells described in this study, the decay times of the sIPSCs (τ_w = 8.5 ± 1.6 ms) were slower than those recorded in α1, β2, γ2 subunit expressing cells. Thus, similar to olfactory bulb granule cells and neurons in the nucleus reticularis thalami, these subunits do not appear to contribute to the postsynaptic $GABA_A$ receptors in juxtaglomerular cells. This is consistent with the lack of α1 subunit mRNA expression in these cells (Laurie et al., 1992).

Furthermore, preliminary results using various concentrations of zolpidem provide further evidence that sIPSCs in juxtaglomerular cells are not mediated by α1 subunit-containing $GABA_A$ receptors. Zolpidem displaces Ro15-1788 from α1β3γ2, α2β3γ2, α3β3γ2 and α5β3γ2 $GABA_A$ receptors with K_i values of 20, 450, 400 and 15000 nM, respectively (Pritchett and Seeburg, 1990). Thus, 50 nM zolpidem should discriminate between α1 and α2/α3/α5 subunit-containing $GABA_A$ receptors. In cerebellar stellate cells, 50 nM zolpidem significantly increased (~170% of control) the decay time of the mIPSCs, but resulted in no significant change in juxaglomerular cells (105% of control). However, 1 μM zolpidem caused a 78% increase of the weighted decay time of sIPSCs in juxtaglomerular cells, indicating that α2/α3 subunit-containing $GABA_A$ receptors mediate these events. These results are consistent with the suggestion that distinct $GABA_A$ receptor subtypes render unique 'kinetic fingerprints' to IPSCs (Puia et al., 1994; Tia et al., 1996a; Lavoie et al., 1997; Brickley et al., 1999; Nusser et al., 1999).

The complete disappearance of spontaneous IPSCs in the presence of TTX revealed the lack of action potential-independent miniature IPSCs in these cells. In most nerve cells studied, mIPSCs are readily observed in the presence of TTX, and even in the presence of the Ca^{2+} channel blocker Cd^{2+}, demonstrating external Ca^{2+} influx-independent release of GABA at most nerve terminals (reviewed by Mody et al., 1994). The precise role of the mIPSCs is largely unknown, but one possibility may be that the spontaneous, action potential-independent release of transmitter is needed for the stabilization of the postsynaptic receptor cluster (McKinney et al., 1999). These results indicate that postsynaptic receptor clusters can be maintained without the action potential-independent release of GABA at some GABAergic synapses.

4.2. Variability in the short-term plasticity of GABAergic synapses

The importance of the dynamic properties of the chemical synapses has been widely recognized and several studies have investigated the short-term plasticity of glutamatergic synapses (Abbott et al., 1997; Zador and Dobrunz, 1997). The short-term plasticity of GABAergic synapses has been studied mainly qualitatively by using paired-pulse protocols and finding paired-pulse depression at most connections (Thomson et al., 1996; Tamas et al., 1997, 1998; Caillard et al., 2000). Quantitative characterization of GABAergic synaptic transmission in the neocortex was reported by Gupta et al. (2000). Using multiple cell recordings and a dynamic neurotransmission model, they distinguished the following three patterns of plasticity: mainly facilitating (F1; τ_{rec}/τ_{fac} = 0.1), mainly depressing (F2; τ_{rec}/τ_{fac} = 40), and combined facilitating-depressing patterns (F3; τ_{rec}/τ_{fac} = 2.8). In the present study, two distinct patterns of short-term plasticity of GABAergic transmission was observed. The depressing synapses had a large τ_{rec}/τ_{fac} value (190) and the facilitating-depressing synapses had an intermediate τ_{rec}/τ_{fac} value (0.8). It has been suggested that the pattern of short-term plasticity at a synaptic connection is determined by both pre- and postsynaptic neuronal elements in the neocortex and the hippocampus (Markram et al., 1998; Gupta et al., 2000; Losonczy et al., 2002). Juxtaglomerular cells comprise several cell types, including the glutamatergic external tufted cells and the presumed GABAergic periglomerular cells. The periglomerular cells are also heterogeneous with respect to their axo-dendritic arborisation, neurotransmitter, and calcium binding protein content (Shepherd and Greer, 1990; Kosaka et al., 1998). It

remains to be determined whether the heterogeneity in the short-term plasticity of GABAergic synapses correlates with juxtaglomerular cell populations, or a diversity of synaptic behaviour characterizes a homogeneous cell population.

4.3. Release-independent short-term facilitation at GABAergic synapses

Two out of the 15 GABAergic connections were mediated by single functional release sites. Both connections showed a facilitating-depressing pattern of plasticity and had a relatively low initial P_r (0.21 and 0.32). During 4 stimuli at 20 Hz, a two-fold decrease in P_f was detected. The decrease of the P_f was independent whether or not the preceding stimuli released transmitter (del Castillo and Katz, 1954). It has been suggested that an elevated [Ca^{2+}] in axon terminals is responsible for short-term facilitation (Katz and Miledi, 1968). Assuming that the residual calcium hypothesis of facilitation holds true at these GABAergic synapses, it is suggested that the failure of transmitter release occurred downstream of action potential invasion and calcium influx. Following the first action potential, [Ca^{2+}] at the release site is not sufficiently high to evoke transmitter release, but could 'prime' the release machinery (probably by interacting with synaptotagmin), increasing the probability that Ca entry at the second action potential will succeed to release transmitter (del Castillo and Katz, 1954; Thomson, 2000; Rozov et al., 2001). Investigating the changes in P_r during trains of action potentials at different frequencies might make it possible to determine the time course of the 'primed' state of the releasable synaptic vesicles.

4.4. Postsynaptic receptor desensitisation during repetitive activation

Studying neurotransmission at single site connections allows the direct identification of presynaptic alterations of P_r, and also provides a powerful tool to investigate such fundamental features of the postsynaptic receptors as their number, occupancy, opening probability, and desensitisation (Silver et al., 1996; Auger and Marty, 2000). Due to the relatively short period of stable recordings of the single site evoked responses in the present study and the low initial P_r, I was unable to collect a sufficiently large number of eIPSCs to perform non-stationary fluctuation analysis (NSFA). Therefore, I determined the single channel current with peak-scaled NSFA of sIPSCs and calculated the number of channels open at the peak of the averaged single site responses (~30 and 40 channels, respectively). By calculating the upper limit of receptor opening probability (0.62 and 0.69) and analysing the coefficient of variation of the responses, I conclude that postsynaptic $GABA_A$ receptors are not saturated by the released transmitter at these synapses. An important consequence of the lack of receptor saturation is that a trial-to-trial fluctuation in the transmitter concentration will be reflected by a trial-to-trial variability in the postsynaptic responses. At these GABAergic synapses the number of postsynaptic $GABA_A$ receptors was calculated to be ~40 and ~60, respectively. These numbers are within the range published for other GABAergic synapses (Auger and Marty, 1997, 2000; Nusser, 1999).

My results demonstrate that postsynaptic mechanisms may also contribute to the short-term plasticity at some GABAergic synapses. In both single site connections, there was an average 15% reduction in the postsynaptic potency during 20 Hz stimulations. A postsynaptic receptor desensitisation seems to be the most likely mechanism, as this decrease in the potency was release-dependent. GABA released by the 1st action potential could drive the receptors into a long-lived desensitised state, which may take several tens of milliseconds to exit from (Jones and Westbrook, 1996; but see Mellor and Randall, 2001). If the 2nd action potential arrives within this time period, the released GABA would not activate such desensitised receptors, resulting in smaller postsynaptic responses. By studying postsynaptic responses at identified single release sites in other brain regions it will be possible to determine whether desensitisation of postsynaptic receptors during repetitive synaptic activation is a general feature of most central GABAergic synapses, or whether it is unique to these olfactory bulb synapses.

Acknowledgements

Funds from the Hungarian Science Foundation (T032309), the Howard Hughes Medical Institute, the Wellcome Trust, the Boehringer Ingelheim Fond, and the Japan Science and Technology Corporation are greatly acknowledged. I would like to thank Drs. Attila Losonczy, Istvan Mody, Mark Farrant and Peter Somogyi for their comments on the manuscript.

References

Abbott, L.F., Varela, J.A., Sen, K., Nelson, S.B., 1997. Synaptic depression and cortical gain control. Science 275, 220–224.

Auger, C., Marty, A., 1997. Heterogeneity of functional synaptic parameters among single release sites. Neuron 19, 139–150.

Auger, C., Marty, A., 2000. Quantal currents at single-site central synapses. J Physiol 528, 3–11.

Barnard, E.A., Skolnick, P., Olsen, R.W., Mohler, H., Sieghart, W., Biggio, G., Braestrup, C., Bateson, A.N., Langer, S.Z., 1998. International union of pharmacology. XV. Subtypes of g-aminobutyric acidA receptors: classification on the basis of subunit structure and receptor function. Pharmacol Rev 50, 291–313.

Bianchi, M.T., Haas, K.F., Macdonald, R.L., 2001. Structural determi-

nants of fast desensitization and desensitization-deactivation coupling in GABAA receptors. J Neurosci 21, 1127–1136.

Bier, M., Kits, K.S., Borst, J.G.G., 1996. Relation between rise times and amplitudes of GABAergic postsynaptic currents. J Neurophysiol 75, 1008–1012.

Borst, J.G.G., Lodder, J.C., Kits, K.S., 1994. Large amplitude variability of GABAergic IPSCs in melanotropes from Xenopus laevis: evidence that quantal size differs between synapses. J Neurophysiol 71, 639–655.

Brickley, S.G., Cull-Candy, S.G., Farrant, M., 1996. Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of GABAA receptors. J Physiol (London) 497, 753–759.

Brickley, S.G., Cull-Candy, S.G., Farrant, M., 1999. Single-channel properties of synaptic and extrasynaptic GABAA receptors suggest differential targeting of receptor subtypes. J Neurosci 19, 2960–2973.

Buzsaki, G., Chrobak, J.J., 1995. Temporal structure in spatially organized neuronal ensembles: a role for interneuronal networks. Curr Opin Neurobiol 5, 504–510.

Buzsaki, G., Llinas, R., Singer, W., Berthoz, A., Christen, Y., 1994. Temporal Coding In The Brain. Springer-Verlag), Berlin.

Caillard, O., Moreno, H., Schwaller, B., Llano, I., Celio, M.R., Marty, A., 2000. Role of the calcium-binding protein parvalbumin in short-term synaptic plasticity. Proc Natl Acad Sci USA 97, 13372–13377.

Chen, W.R., Xiong, W., Shepherd, G.M., 2000. Analysis of relations between NMDA receptors and GABA release at olfactory bulb reciprocal synapses. Neuron 25, 625–633.

del Castillo, J., Katz, B., 1954. Statistical factors involved in neuromuscular facilitation and depression. J Physiol 124, 574–585.

Fritschy, J.-M., Mohler, H., 1995. GABAA-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. J Comp Neurol 359, 154–194.

Gupta, A., Wang, Y., Markram, H., 2000. Organizing principles for a diversity of GABAergic interneurons and synapses in the neocortex. Science 287, 273–278.

Haas, K.F., Macdonald, R.L., 1999. GABAA receptor subunit γ2 and δ subtypes confer unique kinetic properties on recombinant GABAA receptor currents in mouse fibroblasts. J Physiol (Lond) 514, 27–45.

Hajos, N., Nusser, Z., Rancz, E.A., Freund, T.F., Mody, I., 2000. Cell type- and synapse-specific variability in synaptic GABAA receptor occupancy. Eur J Neurosci 12, 810–818.

Isaacson, J.S., Strowbridge, B.W., 1998. Olfactory reciprocal synapses: dendritic signaling in the CNS. Neuron 20, 749–761.

Jahr, C.E., Nicoll, R.A., 1982. An intracellular analysis of dendrodendritic inhibition in the turtle in vitro olfactory bulb. J Physiol 326, 213–234.

Jones, M.V., Westbrook, G.L., 1996. The impact of receptor desensitization on fast synaptic transmission. Trends Neurosci 19, 96–101.

Katz, B., Miledi, R., 1968. The role of calcium in neuromuscular facilitation. J Physiol (London) 195, 481–492.

Kosaka, K., Toida, K., Aika, Y., Kosaka, T., 1998. How simple is the organization of the olfactory glomerulus?: the heterogeneity of so-called periglomerular cells. Neurosci Res 30, 101–110.

Laurent, G., 1999. A systems perspective on early olfactory coding. Science 286, 723–728.

Laurie, D.J., Seeburg, P.H., Wisden, W., 1992. The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. II. olfactory bulb and cerebellum. J Neurosci 12, 1063–1076.

Lavoie, A.M., Tingey, J.J., Harrison, N.L., Pritchett, D.B., Twyman, R.E., 1997. Activation and deactivation rates of recombinant GABAA receptor channels are dependent on (-subunit isoform. Biophys J 73, 2518–2526.

Losonczy, A., Zhang, L., Shigemoto, R., Somogyi, P., Nusser, Z., 2002. Cell type dependence and variability in the short-term plasticity of EPSCs in identified mouse hippocampal interneurones. J Physiol (London) 542, 193–210.

Markram, H., Wang, Y., Tsodyks, M., 1998. Differential signaling via the same axon of neocortical pyramidal neurons. Proc Natl Acad Sci USA 95, 5323–5328.

McKernan, R.M., Whiting, P.J., 1996. Which GABAA-receptor subtypes really occur in the brain? Trends Neurosci 19, 139–143.

McKinney, R.A., Capogna, M., Durr, R., Gahwiler, B.H., Thompson, S., 1999. M.Miniature synaptic events maintain dendritic spines via AMPA receptor activation. Nat Neurosci 2, 44–49.

Mellor, J.R., Randall, A.D., 2001. Synaptically released neurotransmitter fails to desensitize postsynaptic $GABA_A$ receptors in cerebellar cultures. J Neurophysiol 85, 1847–1857.

Mody, I., De Koninck, Y., Otis, T.S., Soltesz, I., 1994. Bridging the cleft at GABA synapses in the brain. Trends in Neurosci 17, 517–525.

Nicoll, R.A., 1971. Pharmacological evidence for GABA as the transmitter in granule cell inhibition in the olfactory bulb. Brain Res 35, 137–149.

Nusser, Z., 1999. A new approach to estimate the number, density and variability of receptors at central synapses. Eur J Neurosci 11, 745–752.

Nusser, Z., Naylor, D., Mody, I., 2001. Synapse-specific contribution of the variation of transmitter concentration to the decay of inhibitory postsynaptic currents. Biophys J 80, 1251–1261.

Nusser, Z., Sieghart, W., Mody, I., 1999. Differential regulation of synaptic GABAA receptors by cAMP-dependent protein kinase in mouse cerebellar and olfactory bulb neurones. J Physiol (Lond) 521, 421–435.

Persohn, E., Malherbe, P., Richards, J.G., 1992. Comparative molecular neuroanatomy of cloned GABAA receptor subunits in the rat CNS. J Comp Neurol 326, 193–216.

Poisbeau, P., Williams, S.R., Mody, I., 1997. Silent GABAA synapses during flurazepam withdrawal are region-specific in the hippocampal formation. J Neurosci 17, 3467–3475.

Pritchett, D.B., Seeburg, P.H., 1990. γ-aminobutyric acidA receptor α5-subunit creates novel type II benzodiazepine receptor pharmacology. J Neurochem 54, 1802–1804.

Puia, G., Costa, E., Vicini, S., 1994. Functional diversity of GABA-activated Cl- currents in Purkinje versus granule neurons in rat cerebellar slices. Neuron 12, 117–126.

Rozov, A., Burnashev, N., Sakmann, B., Neher, E., 2001. Transmitter release modulation by intracellular Ca2+ buffers in facilitating and depressing nerve terminals of pyramidal cells in layer 2/3 of the rat neocortex indicates a target cell-specific difference in presynaptic calcium dynamics. J Physiol (London) 531, 807–826.

Schoppa, N.E., Kinzie, J.M., Sahara, Y., Segerson, T.P., Westbrook, G.L., 1998. Dendrodendritic inhibition in the olfactory bulb is driven by NMDA receptors. J Neurosci 18, 6790–6802.

Shepherd, G.M., Greer, C.A., 1990. In: Sheperd, G.M. (Ed.), Olfactory Bulb, 3rd ed. Oxford University Press, New York.

Sieghart, W., 1995. Structure and pharmacology of γ-aminobutyric acidA receptor subtypes. Pharmacological Reviews 47, 181–234.

Silver, R.A., Cull-Candy, S.G., Takahashi, T., 1996. Non-NMDA glutamate receptor occupancy and open probability at a rat cerebellar synapse with single and multiple release sites. J Physiol (London) 494, 231–250.

Singer, W., 1996. The changing face of inhibition. Current Biology 6, 395–397.

Singer, W., 1999. Time as coding space? Curr Opin Neurobiol 9, 189–194.

Tamas, G., Buhl, E.H., Somogyi, P., 1997. Fast IPSPs elicited via multiple synaptic release sites by different types of GABAergic neurone in the cat visual cortex. J Physiol (London) 500, 715–738.

Tamas, G., Somogyi, P., Buhl, E.H., 1998. Differentially interconnected networks of GABAergic interneurons in the visual cortex of the cat. J Neurosci 18, 4255–4270.

Thomson, A.M., 2000. Facilitation, augmentation and potentiation at central synapses. Trends Neurosci 23, 305–312.

Thomson, A.M., West, D.C., Deuchars, J., 1996. Single axon IPSPs elicited in pyramidal cells by three classes of interneurones in slices of rat neocortex. J Physiol (London) 496, 81–102.

Tia, S., Wang, J.F., Kotchabhakdi, N., Vicini, S., 1996a. Developmental changes of inhibitory synaptic currents in cerebellar granule neurons: role of GABAA receptor α6 subunit. J Neurosci 16, 3630–3640.

Tia, S., Wang, J.F., Kotchabhakdi, N., Vicini, S., 1996b. Distinct deactivation and desensitization kinetics of recombinant GABAA receptors. Neuropharmacology 35, 1375–1382.

Traub, R.D., Spruston, N., Soltesz, I., Konnerth, A., Whittington, M.A., Jefferys, G.R., 1998. Gamma-frequency oscillations: a neuronal population phenomenon, regulated by synaptic and intrinsic cellular processes, and inducing synaptic plasticity. Prog Neurobiol 55, 563–575.

Tretter, V., Ehya, N., Fuchs, K., Sieghart, W., 1997. Stoichiometry and assembly of a recombinant GABAA receptor subtype. J Neurosci 17, 2728–2737.

Tsodyks, M.V., Markram, H., 1997. The neural code between neocortical pyramidal neurons depends on neurotransmitter release probability. Proc Natl Acad Sci USA 94, 719–723.

Williams, S.R., Buhl, E.H., Mody, I., 1998. The dynamics of synchronized neurotransmitter release determined from compound spontaneous IPSCs in rat dentate granule neurones in vitro. J Physiol (Lond) 510, 477–497.

Zador, A.M., Dobrunz, L.E., 1997. Dynamic synapses in the cortex. Neuron 19, 1–4.

Zhang, S.J., Huguenard, J.R., Prince, D.A., 1997. GABAA receptor-mediated Cl- currents in rat thalamic reticular and relay neurons. J Neurophysiol 78, 2280–2286.