Electrophysiological Properties of Rat Pontine Nuclei Neurons In Vitro
II. Postsynaptic Potentials

MARTIN MÖCK, CORNELIUS SCHWARZ, AND PETER THIER
Sektion für Visuelle Sensomotorik, Neurologische Universitätsklinik Tübingen, 72076 Tübingen, Germany

Möck, Martin, Cornelius Schwarz, and Peter Thier. Electrophysiological properties of rat pontine nuclei neurons in vitro. II. Postsynaptic potentials. J. Neurophysiol. 78: 3338–3350, 1997. We investigated the postsynaptic responses of neurons of the rat pontine nuclei (PN) by performing intracellular recordings in parasagittal slices of the pontine brain stem. Postsynaptic potentials (PSPs) were evoked by brief (0.1 ms) negative current pulses (10–250 μA) applied to either the cerebral peduncle or the pontine tegmentum. First, excitatory postsynaptic potentials (EPSPs) could be evoked readily from peduncular stimulation sites. These EPSPs exhibited short latencies, a nonlinear increment in response to increased stimulation currents, and an unconventional dependency on the somatic membrane potential. Pharmacological blockade of the synaptic transmission using 6,7-dinitroquinoxaline-2,3-dione and d,l-2-amino-5-phosphonovaleric acid, selective antagonists of the α-amino-3-hydroxy-5-methyl-4-isoxazolpropionate- (AMPA) and the N-methyl-D-aspartate (NMDA)-type glutamate receptors, showed that these EPSPs were mediated exclusively by excitatory amino acids via both AMPA and NMDA receptors. Moreover, the pharmacological experiments indicated the existence of voltage-sensitive but NMDA receptor-independent amplification of EPSPs. Second, stimulations at peduncular and tegmental sites also elicited inhibitory postsynaptic potentials (IPSPs) in a substantial proportion of pontine neurons. The short latencies of all IPSPs argued against the participation of inhibitory interneurons. Their sensitivity to bicuculline and reversal potentials around −70 mV suggested that they were mediated by γ-aminobutyric acid-A (GABA A ) receptors. In addition to single PSPs, sequences consisting of two to four distinct EPSPs could be recorded after stimulation of the cerebral peduncle. Most remarkably, the onset latencies of the following EPSPs were multiples of the first one indicating the involvement of intercalated synapses. Finally, we used the classic paired-pulse paradigm to study whether the temporal structure of inputs influences the synaptic transmission onto pontine neurons. Pairs of electrical stimuli applied to the cerebral peduncle resulted in a marked enhancement of the amplitude of the second EPSP for interstimulus intervals of 10–100 ms. Delays >200 ms left the EPSP amplitude unaltered. These data provide evidence for a complex synaptic integration and an intrinsic connectivity within the PN too elaborate to support the previous notion that the PN are simply a relay station.

INTRODUCTION

In the accompanying paper, we have shown that complex membrane properties modulate the firing behavior of neurons in the rat pontine nuclei (PN), providing the basis for information processing by dynamic output control (Schwarz et al. 1997). The computational capabilities of neurons are, however, also highly dependent on the mechanisms governing the synaptic transmission onto them. The integration of postsynaptic potentials in turn depends on the dendritic membrane properties and the location of synaptic inputs.

It has been well established by early electrophysiological studies that the cortico-pontine projection, the most numerous afferent input to the PN, as well as several subcortical sources excite pontine neurons monosynaptically (Allen et al. 1969, 1975a,b, 1977; Sasaki et al. 1970; Tsukahara and Bando 1970). Although this basic finding was accepted by all sides, other observations gave rise to two important controversies. Although Sasaki et al. (1970) found evidence for an intrinsic excitatory connectivity within the PN based on axon collaterals, the other groups denied its existence, thus emphasizing a pure relay function of the PN (Allen et al. 1969, 1975a,b, 1977; Tsukahara and Bando 1970). A second point of disagreement was the question whether pontine neurons are inhibited synthetically by pontine interneurons or at least by inhibitory afferents. The resolution of these controversies is important because any kind of intrapontine communication, excitatory as well as inhibitory, would have strong implications for the cerebro-ponto-cerebellar transfer of information.

At present very little is known about the transmitter systems involved in the synaptic transmission onto pontine neurons. Several studies have provided indirect evidence that the cortico-pontine projection uses the excitatory amino acid glutamate as a transmitter (Aas et al. 1992; Dori et al. 1992; Huttner and Baughman 1988; Stone 1976; Thangnipon et al. 1983). In addition, immunohistochemical studies have recently revealed γ-aminobutyric acid (GABA)-immunoreactive fibers and neurons within the PN of several mammalian species, suggesting that inhibition may be mediated by GABA (Aas et al. 1989; Border and Mihailoff 1985, 1990; Border et al. 1986; Brodal et al. 1988; Mihailoff and Border 1990; Thier and Koehler 1987). Both glutamate and GABA act via several different receptor types in a variety of brain regions. The pontine neurons have been suggested to be endowed with several subunits of different ionotropic glutamate as well as GABA receptor subtypes (Frithschy and Mohler 1995; Petralia and Wenthold 1992; Petralia et al. 1994a,b). Each of these receptors evokes specific responses in the postsynaptic neuron (reviewed in Nicoll et al. 1990). The question if GABA receptors are expressed and, moreover, which type of receptor may be present is of fundamental importance for a better understanding of the computational potential of pontine neurons. In the present study, we took advantage of the unique stability of intracellular recordings and the ease of drug application provided by in vitro electrophysiological techniques, which allowed us to reveal directly the major physiological and pharmacological

3338
0022-3077/97 $5.00 Copyright © 1997 The American Physiological Society
properties of postsynaptic responses of pontine neurons in the rat.

METHODS

Slice preparation and maintenance, the basic in vitro electrophysiology techniques, data sampling, and analysis methods employed in this study are similar to those described in the accompanying paper (Schwarz et al. 1997). In the following only substantial variations and additional features will be mentioned.

Postsynaptic potentials were evoked by applying negative current pulses of 0.1-ms duration via lacquer-coated tungsten electrodes. Their impedance ranged from 2.5 to 7 MΩ (at 1 kHz). In most experiments, one stimulation electrode was placed into the cerebral peduncle. An additional electrode was placed into the pontine tegmentum during experiments performed to study inhibitory postsynaptic potentials (IPSPs). Square current pulses were generated by a constant current bipolar stimulus isolator (A365R; WPI, Sarasota, FL) and controlled by a 1401 plus interface (Cambridge Electronic Design, Cambridge, UK). If not explicitly noted otherwise, current pulses were delivered at frequencies of 0.075 or 0.15 Hz during data recording. The minimal stimulus intensity required to elicit excitatory postsynaptic potentials (EPSPs) was determined for every individual neuron. To study the intensity dependency of EPSPs, the intensity was increased from these thresholds in 5- or 10-μA steps until spikes were generated, or to maximally 250 μA. In all other experiments, stimulus intensities were used that evoked medium-sized EPSPs (5–10 mV amplitude) at resting membrane potential under control conditions. Reversal potentials were determined by measuring the amplitude of the postsynaptic response at different somatic membrane potentials (SMPs). A regression line was then fitted to the x-y plot of SMP and amplitude: The crossing of the regression line with the x axis (0 mV amplitude) was taken as reversal potential. Postsynaptic potentials were recorded at a sample rate of 10 kHz. For every stimulus intensity or every test membrane potential, five trials were recorded subsequently. Paired-pulse stimulations were performed with interpulse intervals of 10, 20, 50, 100, 200, 500, and 1,000 ms. The sequence of different interpulse intervals was pseudorandom. Ten trials were recorded for every interpulse interval. The interval between individual trials was 14 s.

Drugs added to inhibit synaptic transmission were diluted in artificial cerebrospinal fluid (ACSF) to final concentrations from stock solutions and saturated with 95% O2-5% CO2 before use. We applied 6,7-dinitroquinoxaline-2,3-dione (DNQX) at concentrations of 5 and 25 μM, D,L-2-amino-5-phosphonoveric acid (APV, 100 μM), and bicuculline methiodide (BMI, 50 μM) all obtained from RBI (Natick, MA). The slices were superfused with these drugs for ≈15 min before recording their steady state effects. Recovery was recorded in all experiments as long as the recording was stable enough. Every neuron served as its own control. In some experiments, we varied the Mg2⁺ concentration of the ACSF between 0 (Mg2⁺-free) and 4.5 mM (high Mg2⁺). Note, however, that Mg2⁺-free ACSF may contain residual Mg2⁺ due to contamination of other compounds used (Mayer and Westbrook 1985; Sutor et al. 1987). We did not add or remove other divalent cations to compensate for changes in [Mg2⁺].

RESULTS

To study the properties of postsynaptic responses onto neurons of the rat pontine nuclei, intracellular recordings were performed from a total of 86 cells. They displayed a resting membrane potential (RMP) of −62 ± 5 mV (mean ± SD, throughout the paper unless otherwise stated), an input resistance of 58 ± 21 MΩ, a membrane time constant of 5.0 ± 2.0 ms, a firing threshold of −40 ± 6 mV, a spike amplitude of 70 ± 8 mV, and a spike width of 0.77 ± 0.15 ms. They were all included in the sample described in the accompanying paper (Schwarz et al. 1997).

Stimulus-response properties of EPSPs

The stimulus-intensity dependency of EPSPs of 40 pontine neurons at RMP was determined by applying 0.1-ms negative current pulses at sites in the cerebral peduncle, which include the cortico-pontine fibers. These stimuli typically evoked EPSPs with short latencies (1.5–7 ms), a fast rising phase, and a slower decay to the RMP. To further characterize these EPSPs, several parameters that describe different features of the EPSP shape were calculated. The magnitude of the depolarization is well characterized by the peak amplitude and the time integral, whereas the time course can be described by the time required to decay from peak to half-maximal amplitude (t1/2 EPSP). The latter, however, is critically dependent on a constant timing of the peak. If the peak amplitude is known, the time integral of the EPSP, which is independent of the peak timing, also can be used as a measure of the EPSP length. The rising of the EPSPs was measured by determining the maximal slope. Finally, the time from stimulus onset to the instant of the maximal rising slope was used as an estimate of the latency. This parameter overestimated the true latencies but yielded consistent results which were not obscured by noise. To present original data, the variabilities between different neurons, and the general tendencies, we will show recordings from individual neurons as well as quantified population data (polynomial fits of nth order and 95% prediction intervals) in this and the following sections.

Typically, elevation of stimulus intensity resulted in increasing EPSP amplitudes but did not influence the latency (35 of 40 cells; Figs. 1A and Fig. 2). At minimal stimulus intensities (i.e., intensities required to depolarize the membrane potential by >3 times the standard deviation of the noise, which never exceeded 0.5 mV), the amplitudes ranged between 0.4 and 5.2 mV, maximal slope between 0.3 and 3.6 mV/ms, t1/2 EPSP between 2.6 and 13.4 ms, and the time integral between 0.2 and 7.4 V·s. Nevertheless, even at minimal stimulus intensities EPSPs were elicited reliably at almost every trial. Maximal values achieved with stimulation intensities that kept the membrane potential just below firing threshold were 18.9 mV (amplitude), 14 mV/ms (maximum slope), 27 ms (t1/2 EPSP), and 26.7 V·s (time integral). Note, however, that the increase of amplitude with increasing current amplitude was not uniform. After an initial increase at lowest stimulus intensities, the amplitude reached a plateau at which additional elevation of intensity had only little effect on the amplitude until even higher intensities led to a further increase. Repetition of this characteristic at higher intensities led to a stepwise stimulus-amplitude relationship. Similar relationships also were exhibited by the maximal slope of the rising phase, t1/2 EPSP, and the time integral (Fig. 1A). These stepwise relationships were characteristic for most of the cells showing increased EPSPs with higher stimulus intensities (34 of 35, 2 steps: n = 10; 3 steps: n = 12, 4 steps: n = 12) and therefore, even can be seen in the
average data (Fig. 2). Most probably they indicate a successive recruitment of additional fibers or fiber bundles and, therefore, an increase in the number of active synapses. A spike was only generated after the EPSP had increased over a range of stimulus intensities, indicating that a simultaneous activation of a considerable number of fibers typically is required to reach firing threshold. Suprathreshold stimulations always resulted in the generation of only one spike. A second spike was prevented by the strong medium afterhyperpolarization, which shuttled the late phase of the EPSP, reducing the probability of further spikes. Even with stimulation intensities of ≤1 mA, no more than two spikes were generated. In A and B, as in other figures, the stimulus artifacts are truncated.

Voltage dependency of EPSPs

To study the voltage dependency of EPSPs in pontine neurons, we evoked medium-sized EPSPs (5–10 mV amplitude) by stimulation of the central peduncle while gradually hyper- and depolarizing the membrane potential by intracellular current injection. Because the neurons most likely were to be penetrated at the soma and, therefore, a direct assessment of the membrane potential at synaptic sites was not possible, the measured membrane potential is referred to as the SMP. The EPSPs of 41 of 46 neurons showed an unconventional voltage dependency. Figure 3A demonstrates a typical case. Quantified data of the entire sample are shown in Fig. 3B. The EPSP amplitudes (Fig. 3B) hardly were affected by changing the SMPS, contradicting the expectation of a reversal potential of ~0 mV typical for EPSPs of central neurons (Eccles 1964). This insensitivity of EPSPs could be due to a remote location of the activated synapses inaccessible to somatic current injection. A second explanation, not necessarily excluding the first one, would be a depolarization-dependent activation of additional depolarizing currents compensating for a reduction of the synaptic K⁺/Na⁺ current. An indication that the latter mechanism indeed plays a role in pontine neurons was given by the dramatic elongation of the EPSPs with depolarized SMPS. The decay to half-maximal amplitude (Fig. 3B) and consequently the time integral (Fig. 3B) increased progressively over the entire range of test potentials. On the other hand, the onset latencies (Fig. 3B) were completely independent of the SMP.

Only 5 of 46 PN neurons showed a conventional voltage dependency of their EPSPs (not shown; estimated reversal potentials as determined by linearly extrapolating the data points available: +0.75 ± 1.7 mV). However, also in these

FIG. 1. Stimulus-response properties of pontine neurons. A: electrical stimulation (25–100 µA in 5-µA steps) of afferent fibers in the cerebral peduncle evoked excitatory postsynaptic potentials (EPSPs) in pontine neurons at resting membrane potential (RMP), which increased in amplitude and width with increased stimulus intensities. Superposition of averages (n = 5) emphasize the nonlinearity of increase. Note that EPSPs evoked with 35–45 µA and 65–75 µA, respectively, are almost congruent, whereas the next intensity steps elicited clearly larger EPSPs. Plots (bottom) show the EPSP amplitude, maximal slope, and time integral as means ± SD of 5 individual trials plotted as function of stimulus intensity. B: suprathreshold stimulation. With increasing stimulus intensity the amplitude of individual EPSPs in another pontine nuclei (PN) cell reached the threshold for action potential generation. The action potential (truncated) characteristically was followed by a marked afterhyperpolarization, resulting in a wavy appearance of the average curves. Additional fibers are recruited by increasing stimulus intensity, as in other figures, the stimulus artifacts are truncated.

FIG. 2. Summary data plots for stimulus-intensity dependency of EPSPs in pontine neurons. Parameters of all EPSPs responding to increased stimulus intensity (Fig. 1) (n = 35) are shown as function of multiples of the threshold intensity. To show the general tendency as well as the variations within the sample, polynomial regressions of 10th order (---) and 95% prediction intervals (· · ·) were calculated. For clarity, single data points are not shown (as in the following summary data plots). All parameters (except for the latency) showed a stepwise relationship to the stimulus intensity in single cells (compare with Fig. 1A), resulting in a wavy appearance of the average curves.
neurons, the decaying phase of the EPSP behaved as described earlier: the decay to half-amplitude decreased with hyperpolarization and increased with depolarization.

Pharmacology of EPSPs

The findings described in the previous section, namely the pronounced voltage-dependency of $t_{1/2}$ EPSP and the time integral, closely resemble the characteristics of glutamatergic synapses with both $\alpha$-amino-3-hydroxy-5-methyl-4-isoxiazolepropionic acid (AMPA) - and $N$-methyl-$d$-aspartate (NMDA)-type receptors. To verify the contribution of glutamate receptors in general and in an attempt to disclose the role of different receptor subtypes, we performed experiments using specific blockers of glutamate receptors.

Addition of the AMPA receptor inhibitor DNQX to the ACSF altered the EPSPs in a dose-dependent and reversible manner. At a concentration of 5 $\mu$M, DNQX reduced the amplitude to $37.3 \pm 9.5\%$ (mean \textpm{} SEM) at RMP ($-60.5 \pm 1.7$ mV), whereas 25 $\mu$M resulted in a reduction to $13.4 \pm 3.7\%$ (mean \textpm{} SEM) at RMP ($-60.8 \pm 4.4$ mV). Experiments in which the effects of a selective blockade of AMPA receptors were studied were based on a concentration of 5 $\mu$M DNQX. This restriction was necessary because quinoxaline derivatives like DNQX are known to antagonize the NMDA receptor-associated glycine binding sites at concentrations $\geq 10$ $\mu$M (Hablitz and Sutor 1990). A representative EPSP before (top) and after DNQX application (bottom) is shown in Fig. 4A. At RMP ($-63$ mV), DNQX lowered the EPSP amplitude, altered its shape, and prolonged the latency (from 1.8 to 2.5 ms in this case). The rising phase was clearly slower, and, therefore, the peak amplitude was reached considerably later (Fig. 4A). The EPSP exhibited a marked voltage dependency of both the peak amplitude and the duration under DNQX: depolarization to a SMP of $-56$ mV led to an increase in amplitude and duration as well as a further substantial prolongation of the time to peak. An opposite effect was obtained by hyperpolarization ($-93$ mV).

![Figure 4](http://jn.physiology.org/)

**FIG. 4.** Effects of 6,7-dinitroquinoxaline-2,3-dione (DNQX) and $D$,L-$2$-amino-5-phosphonovaleric acid (APV) on EPSPs evoked by stimulation in the cerebral peduncle (both $45$ $\mu$A). A: averaged EPSP ($n = 5$) before (top) and after (bottom) application of $50$ $\mu$M APV. Similar to DNQX, APV lowered the magnitude of the EPSP, but it was not effective in altering its overall shape. Variations of the SMP during APV application affected the decaying phase of the EPSP especially at hyperpolarized levels. B: combined application of DNQX and APV. Topmost trace: control EPSP elicited by electrical stimulation at sites in the cerebral peduncle ($40$ $\mu$A). This trace as well as the following ones represent an average of $5$ subsequent trials at a RMP of $-63$ mV. Superfusion with $Mg^{2+}$-free artificial cerebrospinal fluid greatly increased and prolonged the EPSP by washout of $Mg^{2+}$ ions blocking the $N$-methyl-$d$-aspartate (NMDA)-gated ion channel. DNQX ($25$ $\mu$M) partly blocked this EPSP. The remaining component displayed a clearly slower rise and decay compared with the control typical for NMDA-mediated responses. In the next step, the EPSP was blocked completely by further addition of $100$ $\mu$M APV. The EPSP returned to control values after $\geq 60$ min washout.
of the EPSP depends critically on a stable latency of the maximum, a requirement that is not fulfilled under DNQX (Fig. 4A). Similar to the example in Fig. 4A, all parameters shown were altered strongly by DNQX. Individual EPSP parameters exhibited marked differences regarding the effect of varying the SMP under DNQX. The maximal slope and the time integral were each reduced by about the same amount independent of the SMP. The latency was prolonged by blocking AMPA receptors (from $2.2 \pm 0.4$ ms to $2.9 \pm 0.5$ ms at RMP, $n = 4$). The amplitude of the remaining EPSP component showed a marked voltage dependency as indicated by the nonlinear steep rise of the amplitude-SMP relationship at somatic potentials of about $-60$ mV (Fig. 5A). These results demonstrate that a substantial component of the EPSPs recorded in pontine neurons is elicited by excitatory amino acids (EAAs) acting via the AMPA-type receptor.

The slow rise and the prominent voltage dependency of the EPSP component after DNQX application is reminiscent of responses mediated by the second class of glutamate receptors, the NMDA receptor (Nowak et al. 1984). To test if NMDA receptors are indeed involved, the selective NMDA receptor inhibitor APV (100 µM) was applied. As shown in Fig. 4B, the amplitude of the EPSP was reduced reversibly at RMP after APV application without affecting the general shape of the EPSP. Note, however, that APV did not completely abolish the voltage sensitivity of the EPSP. In contrast to the amplitude, which was not affected very much by hyper- or depolarization, the decay from peak still was altered strongly by varying the SMP (Fig. 4B). Quantified data describing the entire sample are presented in Fig. 5B. APV reduced both amplitude and maximal slope by an almost constant value compared with the control over the entire range of test potentials. Compared with the effect of DNQX (Fig. 5A), the reduction of the mean amplitude at RMP was smaller (to $60.4 \pm 20.1\%$; $n = 4$). Most importantly, no increase of EPSP amplitude was seen with depolarization under APV, a feature that was prominent under DNQX. As demonstrated by the time integral, other voltage-dependent processes were reduced by APV as expected but nevertheless still were visible. The integral was enhanced almost 10-fold by changing the SMP from $-95$ to $-45$ mV under APV. Because the amplitude and maximal slope under APV were almost not modulated (or even tended to decrease) with different SMPs, this increase in time integral was mostly due to a prolonged EPSP decay, supporting our observation in single potential traces (see Fig. 4B). Therefore, most likely, NMDA as well as non-NMDA voltage-
dependent processes contribute to the decay and thus the duration of the EPSP. Taken together these results show that EAAs contribute to the synaptic transmission onto pontine neurons, acting via AMPA as well as NMDA receptors. On the other hand, the data presented so far do not rule out the involvement of additional transmitter systems. This possibility was tested by simultaneously blocking both AMPA and NMDA receptors in five neurons (Fig. 4C). To facilitate the detection of the NMDA component of the control EPSP at RMP, in a first step Mg\^{2+} largely suppressing the NMDA response at RMP was removed from the ACSF. This resulted in an increase in EPSP amplitude due to NMDA receptors. Adding a high concentration of DNQX (25 \(\mu\)M) greatly reduced the EPSP without abolishing it completely. The amplitude was reduced to 30.6 ± 11.2\%, the slope to 19.7 ± 15.1\%, and the time integral to 49.3 ± 6.3\%. In contrast, the latency and \(t_{1/2}\) were prolonged to 187.8 ± 6.6\% and 152.6 ± 44.5\%, respectively (\(n = 5\)). Finally, the addition of 100 \(\mu\)M APV completely suppressed the EPSP. The same result was obtained in all five cases tested, indicating that the excitatory synaptic transmission was mediated exclusively by EAAs.

**IPSPs**

In two (of 25) cases the pharmacological blockade of EAA-mediated EPSPs unmasked inhibitory postsynaptic potentials (IPSPs) evoked by electrical stimulation at sites in the cerebral peduncle. IPSPs also were (\(n = 7\)) observed occasionally after very-low-intensity stimulation of the peduncle even in the absence of blockade of the EAA-mediated transmission. An example is presented in Fig. 6A. Stimulation under control conditions evoked an EPSP (top). Addition of 25 \(\mu\)M DNQX, supposed to block completely the AMPA receptor and partially antagonize the NMDA receptor, was sufficient to reverse the direction of the postsynaptic response. Note that the latencies of both EPSP and IPSP were identical. The nature of the inhibitory transmitter and its postsynaptic receptors contributing to inhibition of pontine neurons were disclosed pharmacologically. The specific GABA\(_{\text{A}}\) receptor inhibitor BMI (50 \(\mu\)M) completely and reversibly blocked these IPSPs evoked by peduncular stimulation (Fig. 6A). Furthermore we found that the reversal of the IPSPs corresponded closely to the value expected for GABA\(_{\text{A}}\) receptors. IPSPs elicited by peduncular stimulation had reversal potentials of \(-69.8 ± 3.0\) mV, a value characteristic for the GABA\(_{\text{A}}\) receptor-associated chloride channel (Kaila 1994; Nicoll et al. 1990). A multitude of possible extrinsic sources for inhibition of pontine neurons in the rat have been proposed including GABAergic neurons located in the pontine tegmentum outside the PN (Border et al. 1986; Mihailoff et al. 1992). Our slice preparation enabled us to test this possibility by applying electrical stimuli at various sites within the pontine tegmentum. Although many of these attempts were ineffective, we obtained recordings from three neurons in which IPSPs were evoked by tegmental stimulation. One of them is shown in Fig. 6B. Similar to IPSPs elicited by peduncular stimulation, they were blocked completely by BMI and reversed at \(-70.7 ± 1.0\) mV (Fig. 6C), suggesting that they were GABA\(_{\text{A}}\) receptor-mediated as well.

**Sequences of PSPs**

Electrical stimulation at sites in the cerebral peduncle frequently yielded multiphasic EPSP sequences in PN neurons.
As shown in Fig. 7A, these sequences in most cases consisted of two clearly distinguishable EPSPs, which where both strikingly voltage dependent as indicated by a remarkable shortening by hyperpolarization from RMP. The peak-to-peak latency was 3 ms in this example and ranged up to 6 ms in others. Characteristically, the latency from the stimulus to the onset of the second (“late”) EPSP was about twice, sometimes even three times, as long as that of the first EPSP. To determine whether these EPSP sequences were mediated by the same transmitter receptors as the single EPSPs, their sensitivity to DNQX, APV, or variations in Mg\(^{2+}\) concentration was tested \((n = 4)\). In the example presented in Fig. 7A, we first perfused the slice with Mg\(^{2+}\)-free ACSF. This treatment led to an amplitude enhancement of both the early and the late EPSP at RMP, probably as a consequence of the removal of the Mg\(^{2+}\) block of NMDA receptor-gated ion channels. Addition of the AMPA receptor inhibitor DNQX \((25 \mu M\) in this case\) profoundly reduced the amplitude, resulting in a slowly rising, long-lasting EPSP that was no longer composed of distinguishable components. The disappearance of the second component under DNQX suggests that the late EPSP may have been generated via an intercalated neuron that stopped firing due to the blockade of the AMPA receptors. The contribution of NMDA receptor-gated conductances to the synaptic transmission was confirmed by the complete blockade of the remaining EPSP by increased Mg\(^{2+}\) concentration in the bathing medium \((4.5 \, \text{mM})\) in the presented example or by application of 100 \(\mu M\) APV in the remaining three cases. The EPSP sequences recorded in pontine neurons exhibited nonuniform stimulus-intensity dependencies, which are illustrated in Fig. 7, B and C. Usually single EPSPs occurred at low-stimulus intensities that, however, considerably differed in their latencies, ranging from \(~2\) to 7 ms. These single EPSPs increased with higher stimulus intensities, like those shown in Fig. 1, until at differing intensities the threshold for the generation of a second EPSP was reached. In the majority of recordings \((n = 16\) of 20\), the early EPSP was elicited at low intensities, whereas the late EPSP required higher intensities \((\text{Fig. 7B})\). Once the threshold was reached, the multiphasic EPSP could be recorded consistently in every trial in 12 cases of 20. In the remaining cases, however, multiphasic \((\text{Fig. 7B, black trace})\) as well as single EPSPs \((\text{Fig. 7B, gray trace})\) occurred alternately at the same stimulus intensity. It is noteworthy that we found 4 EPSP sequences out of 20 in which the late EPSP was elicited at substantially lower stimulus intensities than the early one \((\text{Fig. 7C})\).

An EPSP sequence consisting of more than two individual EPSPs is shown in Fig. 7, D and E. Close examination of the first 10 ms after stimulus onset at a higher temporal resolution \((\text{Fig. 7E, top})\) disclosed four subsequent EPSPs. This is also evident in the differential \((\text{Fig. 7E, bottom})\), where four peaks indicating the times of local slope maxima of the EPSPs can be discerned \((\cdots\cdots)\). The peaks were used as estimates of the latencies. Note that the latencies of the second to fourth EPSP were approximate multiples of the first one. Indeed, normalizing the stimulus-onset latencies of the secondary EPSPs in the 20 cells showing EPSP sequences with respect to the onset latency of the first EPSP \((\text{Fig. 7F})\) reveals that, as a rule, the onset latencies of second, third, or fourth EPSPs correspond to approximate multiples of that of the first one. Even cell 20, seemingly most deviating from the rule, might reflect this periodicity, because the long latency of the first EPSP \((3.7\) ms in this case\) suggested that it may have been generated disynaptically, whereas a directly mediated one with a correspondingly shorter latency failed to be evoked.

### Effects of paired-pulse stimulation

The synaptic transmission onto many neurons is not invariant over time especially for repetitive stimulations, but depends on preceding events. To study if the temporal structure of inputs impinging on pontine neurons alters the synaptic transmission, we applied pairs of electrical stimuli with variable delays at sites in the cerebral peduncle \((n = 10)\). These delays ranged from 10 to 1,000 ms corresponding to frequencies of 100 to 1 Hz. Possible effects of synaptic facilitation or depression were monitored by comparing the amplitudes of the EPSPs evoked by the two stimuli. The recording exhibiting the most prominent changes by paired-pulse stimulation compared with single-pulse stimulation is presented in Fig. 8. To quantify amplitude alterations, the first EPSP was subtracted from the second one. Because at higher stimulus frequencies partial overlap occurred, we subtracted an average of control EPSPs evoked by standard stimuli in the case of stimulus frequencies \(>10\) Hz. By this procedure, the second EPSP was obtained in isolation for all stimulus frequencies \((\text{Fig. 8B, left trace represents the control})\). Finally, the pure amplitude changes were calculated \((\text{Fig. 8C})\). In this example, paired stimulation at 1 and 2 Hz did not enhance synaptic transmission. Facilitation, however, occurred at frequencies \(\approx 5\) Hz, with a maximal enhancement to 220\% of the control value at 50 Hz. The results obtained from all 10 neurons are summarized in Fig. 8D. Because the data were not normally distributed they are given as median \((\text{horizontal bar})\), 25–75\% quartiles \((\text{box})\), and 100\% range \((\text{vertical bars})\). In general, paired-pulse facilitation occurred in all neurons tested. Moreover, the frequency dependency of this short-term synaptic enhancement was similar in all cases. Although paired stimulation at frequencies of 1 or 2 Hz produced no facilitation, increasing the frequency to 5, 10, or 100 Hz resulted in clearly elevated amplitudes in most cases. However, at these frequencies, individual cases ranged around the control value. In contrast, stimulations at 20 and 50 Hz reliably induced paired-pulse facilitation. The significance of amplitude enhancement by paired stimuli was tested for all frequencies using the Wilcoxon matched pairs test. As indicated by asterisks in Fig. 8D, facilitation was significant for 5 Hz stimulations \((P < 0.025)\) and for 10–100 Hz \((P < 0.02)\). Stimulations at 50 and 100 Hz exhibited highly scattered results as compared with 20-Hz stimulations. In parallel, the median values clearly decreased with very short interstimulus intervals. To test whether this phenomenon might be caused by coactivation of inhibitory inputs, we repeated the experiment after BMI application \((50 \mu M)\) in two cases. Blockade of GABA\(_A\) receptor-mediated inhibition in fact selectively increased the facilitation induced by 50- and 100-Hz stimulation to the level of those observed at 20 and 10 Hz.
FIG. 7. Multiphasic EPSPs. A: typical example of an EPSP sequence evoked by a single pulse (80 µA) applied in the cerebral peduncle. All traces are averages (n = 5). At RMP (−63 mV), 2 EPSPs occurred with a peak-to-peak latency of 3 ms. Hyperpolarization to −86 mV SMP emphasized the distinctness of both EPSPs due to a faster decay. Removal of Mg²⁺ ions from the bath solution at RMP increased both EPSPs in amplitude and length. Blocking AMPA receptors and the glycine binding site of the NMDA receptor by 25 µM DNQX lowered the amplitude without influencing the length of the EPSP sequence. After blocking AMPA receptors, only the early EPSP remained. That the remaining EPSP was NMDA receptor-mediated is confirmed by the lowest trace showing that elevated Mg²⁺ concentration in addition to DNQX entirely blocked both EPSPs. B and C: stimulus-intensity dependency of multiphasic EPSPs. In B, low-intensity stimulation (60 µA) evoked single EPSPs with short latencies. After enhancement of the intensity to 80 µA, a 2nd EPSP was generated with a peak-to-peak latency of 2 ms. At this threshold intensity, however, not every stimulation yielded multiphasic EPSPs. Frequently single (gray trace) and multiphasic EPSPs (black trace) alternated. On the other hand in a few examples (C), the EPSP observed at low intensities (130 µA) had substantially longer latencies (6 ms in this case). Increasing the stimulus intensity to 210 µA also evoked an additional EPSP but, in contrast to B, this one occurred at a latency of only 3 ms. D: average of 5 subsequent trials showing the most complex EPSP sequence recorded in pontine neurons after a single electrical stimulation in the cerebral peduncle (170 µA). In addition to the obvious late EPSP rising with a latency of ~7.5 ms, there were clear inflections during the initial rise of the entire complex not observed in any other neuron. E: illustration of the 1st 10 ms after stimulation (dashed box in A) of the same multiphasic EPSP (top) and the corresponding differential (bottom). Rising phase of the EPSP sequence was composed of 3 distinct single EPSPs, thus the entire complex consisted of 4 EPSPs. Differential exhibits 4 local maxima, each one closely matching the rising of individual single EPSPs (vertical dashed line). Remarkably, they were spaced almost equally except for the last one, which, however, had a twice as long delay. F: periodicity of EPSP sequences. Latencies from stimulus onset to the onset of individual EPSPs within a EPSP sequence were determined manually at stimulus intensities consistently evoking a constant number of EPSPs. They were normalized with regard to that of the 1st one, and means ± SD (n = 5) were plotted for each EPSP in the sequence. In general, the latencies of pursuing EPSPs turned out to be precisely or close to multiples of the 1st one. Only 1 case (cell 20) clearly deviated from this relationship.
FIG. 8. Paired-pulse facilitation. A: averaged postsynaptic response \( (n = 10) \) recorded in a pontine neuron after paired stimulation in the cerebral peduncle \( (100 \, \mu A, 20\text{-ms interpulse interval}) \). The conditioning pulse markedly facilitated the response to the 2nd one as indicated by a more than 2-fold increase in peak amplitude. B: averaged control \((\text{left})\) and 2nd EPSPs for the entire range of stimulus frequencies \((100–1 \, \text{Hz}, \text{arranged according to C})\) recorded in the same neuron as A. Isolated 2nd EPSPs were obtained by subtracting the control from EPSP pairs. At high pulse frequencies, a substantial enhancement of the synaptic transmission is obvious. C: changes in amplitude produced by paired stimulation for all frequencies used \((\text{given at the bottom})\). They were determined by subtracting the control from the isolated 2nd EPSPs depicted in B. In this case, paired-pulse facilitation occurred at frequencies between 100 and 5 Hz. Lower frequencies had neither facilitating nor depressing effects. D: summary of paired-pulse experiments \((n = 10)\). Percentage of amplitude changes as function of the stimulation frequency is shown as box-and-whisker plot. Medians are given by horizontal bars, 25–75% quartils by boxes, and the range by vertical bars. In all experiments, the results obtained by 1- and 2-Hz stimulations scattered around their control values. In contrast, higher frequencies consistently resulted in increased medians with a maximum at 20 Hz. Facilitation was tested for significance using the Wilcoxon matched pairs test. Amplitudes of the 2nd EPSPs were increased significantly for stimulus frequencies of 5–100 Hz at error levels of \( P < 0.025 \) \((*)\) or \( P < 0.020 \) \((**), \) respectively. The drop in median and the increase in variability observed at 50 and 100 Hz may be due to interactions with coactivated inhibitory inputs in some experiments.

DISCUSSION

In the present study, we demonstrated that the excitatory synaptic transmission onto neurons of the PN is purely glutamatergic, involving both AMPA- and NMDA-type receptors, and that it is subject to a robust, frequency-dependent short-term enhancement. The characteristics of the EPSPs suggest a location of most excitatory synapses remote from the soma. We will discuss that voltage-gated synaptic and nonsynaptic conductances contribute to the spread of these EPSPs and that intrinsic axon collaterals may be the cause of multiphasic EPSPs occasionally obtained. Finally, we will discuss the evidence for GABA\(_\alpha\) receptor-mediated inhibition in a substantial proportion of PN neurons.

Excitation of pontine neurons

The excitatory responses observed were all evoked by low-intensity stimulations at sites in the cerebral peduncle. In the rat, a multitude of subcortical structures project to the PN (Mihailoff et al. 1989) and intersect the cerebral peduncle before they terminate. Nevertheless, it is most likely that the vast majority of EPSPs were elicited via cortico-pontine fibers, as they outnumber other afferent fibers by far. In rats, the number of subcortical afferent neurons identified by retrograde transport of wheat germ agglutinin-horseradish peroxidase is very small even after large injections into the PN (Mihailoff et al. 1989), contrasting with the dense labeling obtained in all parts of the rat neocortex (Legg et al. 1989). More quantitative studies in the cat reported that subcortical fibers constitute only 10% of all pontine afferents (Aas 1989).

In the present study, we were able to block pontine EPSPs using specific EAA receptor antagonists suggesting that EAAs are involved in the cortico-pontine transmission. This finding confirms previous studies that provided more indirect evidence for glutamatergic transmission with a variety of methods. First, pharmacological experiments using prelabeled cortico-tectal neurons in culture revealed that the cor-
tico-tectal projection in the rat is in fact glutamatergic (Huet
tner and Baughman 1988). Because the cortico-tectal and the
cortico-pontine projections arise at least partially from the
same cells (Wang and McCormick 1993), it follows that
at least a part of the cortically derived excitation of pontine
neurons also is mediated by glutamate. Second, a substantial
part of layer V pyramidal cells in rat visual cortex retro-
gradely labeled from the PN exhibits glutamate immunoreac-
tivity (Dori et al. 1992), and fibers of the pyramidal tract,
some of which send collaterals to the PN, were suggested
to form glutamatergic synapses (Stone 1976). Third, inter-
ruption of the cortico-pontine pathway reduces the high-
affinity uptake of glutamate by the PN (Thangnipon
et al. 1983) and the release of glutamate in the PN by sus-
tained potassium-induced depolarization (Aas et al. 1992).
Finally, several subunits of different glutamate receptor
types were localized immunohistochemically on pontine
neurons (Petralia and Wenthold 1992; Petralia et al.
1994a,b). In the present study, IPSPs (2 of 25) were detected
occasionally after applying antagonists of glutamatergic
transmission. The scant number and the absence of large
shunting effects (as discussed below) renders it unlikely that
the parameters of EPSPs reported here were significantly
affected by IPSPs. In line with this notion, seven EPSP tested
under BMI showed no significant change in their parameters
to the control values.

Our pharmacological experiments revealed that both
AMPA and NMDA receptor subtypes are involved in cort-
tico-pontine synaptic transmission. As shown in other prepa-
rations of brain tissue (Andreasen et al. 1989; Blake et al.
1988; Honoré et al. 1988), DNQX reduced the amplitude
of pontine EPSPs but failed to block them entirely even at
high doses. A slowly rising and long-lasting EPSP persisted
with respect to stimulation intensity. The relationship be-
tween stimulus strength and EPSP amplitude, however, was
steplike, indicating that the afferents to the recorded neuron
were not distributed continuously with respect to the stimu-
lus electrode. We hypothesize that this characteristic
is achieved by successive activation of distinct fiber bundles.
In the only previous report of a systematic attempt to record
EPSPs of cortico-pontine synapses intracellularly (Allen et
al. 1977), a similar intensity response relationship was
described for pontine neurons in the cat in vivo.

Our finding of mostly unchanged or increased EPSP am-
plitudes with depolarized SMPs is not compatible with the
conventional notion that EPSP amplitudes show an equilib-
rium potential near 0 mV ( Eccles 1964). One possible expla-
nation of this phenomenon is that the EPSPs may be gener-
ated by synapses formed on distal parts of the dendritic tree
and thus substantially attenuated and slowed in time course
due to the passive membrane properties (Jack et al. 1983;
Rall 1977). In fact, electron microscopic analysis of synaptic
terminals in PN of different species revealed support for
this possibility ( Cooper and Beal 1978; Holländer et al.
Only a small portion of the boutons were shown to contact
the soma, whereas the majority turned out to be axodendritic.
Most noteworthy, almost no synapses were formed on large-
diameter proximal dendrites. Instead, most synapses were
observed on small-diameter distal dendrites and dendritic
appendages. A recent study in the rat using intracellular
filling demonstrated that dendritic appendages indeed are
located predominantly on the distalmost parts of dendrites
(Schwarz and Thié 1996).

Besides the distal dendritic location of synaptic inputs,
several additional mechanisms have been discussed to con-
tribute to unconventional voltage dependencies. In addition
to NMDA-mediated currents as stated above, clearly, other
ductances contributed to the boosting of EPSPs as was
shown by the remaining voltage dependency of the EPSP
integral during blockade of NMDA receptors (Figs. 4 and 5)
(Sutor and Hable 1989). At present two inwardly directed
currents are discussed for this function: a persistent sodium
current (reviewed in Crill 1996) and the low-threshold cal-
cium or T current (reviewed in Huguenard 1996), both
known to amplify EPSPs spreading from distal dendritic
sites to the soma (Deisz et al. 1991; Schwindt and Crill
1995; Stuart and Sakmann 1995). Based on the present data,
it is not possible to definitively attribute the observed
effects in pontine neurons to one of these currents. There is
clear evidence given in the accompanying paper that a persist-
ent sodium current is present in PN neurons (Schwarz et al.
1997), whereas no indication for the presence of the T cur-
rent as, e.g., rebound spiking after hyperpolarizing intracel-
lular stimulations, was obtained. Finally, it has to be men-
tioned that a mechanism acting on metabotropic glutamate receptors with subsequent reduction of outward potassium currents, not addressed by the present experiments, cannot be ruled out at present (Charpak et al. 1990; Wang and Daw 1996).

An additional prominent feature of the synaptic transmission onto rat pontine neurons is a strong paired-pulse facilitation for inputs arriving within ≈100 ms. A similar potentiation was described previously in the cat in vivo (Allen et al. 1977) but for interstimulus intervals of only 2–10 ms. The temporal range for short-term enhancement at the cortico-pontine synapse is, therefore, considerably longer than believed before. This mechanism may add to the capability of pontine neurons to preferentially detect and convey specific temporal patterns in their inputs.

Inhibition of pontine neurons

One of the main arguments challenging the view that the PN simply relay cerebral information to the cerebellum derives from studies proposing intrinsic inhibition. In contrast to the evidence provided by extensive morphological studies in different species (Aas et al. 1989; Border and Mihailoff 1985, 1990; Border et al. 1986; Brodal et al. 1988; Mihailoff and Border 1990; Thier and Koehler 1987), unequivocal physiological and pharmacological data are lacking or are at least a matter of debate (Allen et al. 1975b, 1977; Berretta et al. 1991; Sasaki et al. 1970). In the present study, we were able to demonstrate that rat pontine neurons indeed receive inhibitory GABAergic inputs via GABA$_A$ receptors. Moreover, the finding that IPSPs were elicited in >20% of the neurons (12 of 55), 3 of 12 tested by stimulation of sites in the pontine tegmentum the other 9 by stimulation within the cerebral peduncle, implies that inhibition is of general importance. We suppose that this percentage probably underestimates the true impact of inhibition because missing the appropriate stimulation sites may mask the true proportion. The properties of the IPSPs recorded are in accordance with some of the morphological features of the rat’s PN. The latencies of the IPSPs indicate monosynaptic connections. Because the stimulation sites were extrinsic, it seems unlikely that GABAergic interneurons within the PN were involved. This conclusion corresponds to the remarkably low number of reported GABA-immunoreactive pontine neurons in rat (Border and Mihailoff 1985, 1990; Brodal et al. 1988). GABAergic afferent systems have been reported to be especially prominent in the rat PN, possibly compensating for the lack of GABAergic interneurons (Border et al. 1986). A characteristic feature of the synaptic responses recorded in the present study was that EPSPs were detectable even when inhibitory fibers were stimulated simultaneously. Accordingly, inhibitory synapses do not seem to be located in a position allowing them to shunt all excitatory inputs. Electron microscopic studies of GABA-immunoreactive terminals in the rat indeed revealed that >90% of all putative inhibitory synapses were formed on dendrites or dendritic appendages and only a very small portion at the soma (Border and Mihailoff 1990). Additional support for this view comes from immunohistochemical localization of GABA$_A$ receptors in the neuropil of the rat PN while being mostly absent from the somata (Fritschy and Mohler 1995).

Intrinsic connectivity within the pontine nuclei

In the present study, ~23% of the EPSPs (20 of 86 neurons) consisted of two to four distinct components with latencies of the later components being multiples of the latency between stimulus and first EPSP. This points to intrinsic excitatory connections within the slice. The probability that these multisynaptic connections are conveyed via neurons in the reticular formation is small as the projection of the reticular formation to the PN is weak (Mihailoff et al. 1989) and the distance to the recorded neurons in the ventral parts of the PN is quite large. This strongly suggests that these connections are intrinsic to the PN. Consistent with this finding is evidence from a retrograde degeneration study that pontine projection neurons may have axon collaterals intrinsic to the PN (Mihailoff 1978). This view also was maintained by Sasaki et al. (1970), who observed periodic activity of single units in the cat PN using antidromic activation of pontine neurons from the contralateral cerebellar hemisphere in vivo. Allen et al. (1975b) also described EPSP sequences in the cat after stimulation in the subcortical white matter, the internal capsule, the cerebral peduncle, and the medullary pyramid in vivo. However, they attributed the individual components of these EPSP sequences to different corticofugal fibers terminating on one single pontine neuron and conveying action potentials with different velocities. For several reasons, we do not agree with this notion but rather propose that these EPSP sequences arise from intrinsic connections. First, the intervals between individual EPSPs in their recordings are compatible with synaptic delays expected from intrinsically evoked EPSPs. Furthermore, these intervals were not subject to alterations dependent on the site of stimulation as would be expected if different conductance velocities were the cause of the EPSP sequences (Allen et al. 1975b, see their Fig. 5). Second, our finding of multiphasic EPSPs in the slice cannot be explained by differences in conductance velocity. For example, with the extreme conductance velocities measured in the study of Allen et al. (4 and 80 m/s), the consecutive latency difference would be in the range of ~200 μs considering the short distance of the stimulation electrode from the synaptic site in the slice (maximally 1 mm).

At present, we are far from a detailed knowledge about the putative intrinsic connectivity within the PN. However, because the most important afferent (Angaut et al. 1985; Brodal 1978, 1987; Lee and Mihailoff 1990; Mihailoff et al. 1985; Watt and Mihailoff 1983; Wiesendanger and Wiesendanger 1982) and efferent systems (Mihailoff et al. 1981; Nikundiwe et al. 1994) of the PN are characterized by a divergent and patchy organization, an intrinsic connectivity based on axon collaterals may have implications for the way the PN process and distribute information destined for the cerebellum. In view of our previous finding that the compartments within the PN are well segregated from each other (Schwarz and Thier 1995), the most important question is whether intrapontine communication is restricted to individual termination zones or if alternatively different termination zones of the same or even different cortical regions are interconnected by pontine axon collaterals. Our findings in the slice preparation, which necessarily reduces the probability for the detection of relatively long-range connections within...
the PN, favor a localized communication. However, besides locally arborizing axons, putative axon collaterals also were supposed to arise at some distance from the cell soma (Mihailoff 1978). In addition, the percentage (36%, 68 of 189 neurons) of multiphasic EPSPs recorded in vivo (Allen et al. 1975b) was considerably higher (significant at an error level of <0.01, χ² test) than in our study (23%, 20 of 86 neurons). Therefore, functional coupling by means of axon collaterals potentially occurs between both adjacent and distant pontine neurons, opening a variety of possibilities to modulate information transfer through the PN.

We thank U. Grosshennig for excellent technical assistance and D. Plackontakis for improving our English.

This work was supported by Deutsche Forschungsgemeinschaft Grant SFB 307-A1. M. Mock was supported partly by fortunate (Medical School, University of Tubingen, Germany).

Address for reprint requests: C. Schwarz, Sektion für visuelle Sensomotorik, Neurologische Universitätsklinik Tübingen, Hoppe-Seyler Strasse 3, 72076 Tübingen, Germany.

Received 9 October 1996; accepted in final form 29 July 1997.

REFERENCES


McDermott, A. B. and Dale, N. Receptors, ion channels and synaptic...
polarizations underlying the integrative actions of excitatory amino acids. 

Mihailoff, G. A. Principal neurons of the basilar pons as the source of a 

Mihailoff, G. A. and Border, B. G. Evidence for the presence of presyn-
aptic dendrites and GABA-immunogold labeled synaptic boutons in the 

Mihailoff, G. A., Kosinski, R. J., Azzi, S. A., and Border, B. G. Survey of 
noncortical afferent projections to the basilar pontine nuclei: a retro-

Mihailoff, G. A., Kosinski, R. J., Azzi, S. A., Lee, H. S., and Border, 
B. G. The expanding role of the basilar pontine nuclei as a source of 
cerebellar afferents. In: The *Cerebellum Revisited*, edited by R. Llinás and 

basilar pontine nuclei from face sensory and motor regions of the cerebral 

Mihailoff, G. A. and McArule, C. B. The cytoarchitecture, cytology, 
and synaptic organization of the basilar pontine nuclei in the rat. II. 

Mihailoff, G. A., Wett, C. B., and Byrne, R. A. Evidence suggesting 
that both the corticopontine and the cerebellopontine systems are each 
composed of two separate neuronal populations: an electron microscopic 
and horseradish peroxidase study in the rat. *J. Comp. Neurol.*, 195: 221– 
242, 1981.

Miles, R. and Wong, R.K.S. Excitatory synaptic interactions between CA3 
neurons in the guinea-pig hippocampus. *J. Physiol. (Lond.*)* 373: 397– 
418, 1986.

Nicoll, R. A., Malekna, R. C., and Kauer, J. A. Functional comparison of 
neurotransmitter receptor subtypes in mammalian central nervous sys-

Nikundwe, A. M., Bjaalie, J. G., and Brodal, P. Lamellar organization 
of pontocerebellar neuronal populations. A multi-tracer and 3-D computer 

Nowak, L., Breghestovski, P., Ascher, P., Herrett, A., and Prochiantz, 
A. Magnesium gates glutamate-activated channels in mouse central neu-

Oka, H., Sasaki, K., Matsuda, Y., Yasuda, T., and Mizuno, N. Re-
sponses of pontocerebellar neurons to stimulation of the parietal associa-

Petralia, R. S. and Wenthold, R. J. Light and electron immunocyto-
chemical localization of AMPA-selective glutamate receptors in the rat 

Petralia, R. S., Wang, Y.-X., and Wenthold, R. J. Histological and 
ultrastructural localization of the kainate receptor subunits, KA2 and 
Glut/7, in the rat nervous system using selective antipeptide antibodies. 

Petralia, R. S., Yokotani, N., and Wenthold, R. J. Light and electron 
microscope distribution of the NMDA receptor subunit NMDAR1 in the 
rat nervous system using a selective antipeptide antibody. *J. Neurosci.* 

Rall, W. Core conductor theory and cable properties of neurons. In: *Hand-

Ruegg, D. and Wiesendanger, M. Corticofugal effects from sensimotor 
area I and somatosensory area II on neurons of the pontine nuclei in 

Sasaki, K., Kawaguchi, S., Shimoto, T., and Prelevic, S. Electrophysio-

Schwarz, C., Mock, M., and Thier, P. Electrophysiological properties of 
rat pontine nuclei neurons in vitro. I. Membrane potentials and firing 

Schwarz, C. and Thier, P. Modular organization of the pontine nuclei: 
dendritic fields of identified pontine projection neurons in the rat respect 

Schwarz, C. and Thier, P. Comparison of projection neurons in the pont-
ine nuclei and the nucleus reticularis tegmenti pontis of the rat. *J. Comp. 

Schwendt, P. C. and Chill, W. E. Amplification of synaptic current by 
persistent sodium conductance in apical dendrites of neocortical neurons. 

Stone, T. W. Blockade by amino acid antagonists of neuronal excitation 

Stuart, G. and Sakmann, B. Amplification of EPSPs by axosomatic 
sodium channels in neocortical pyramidal neurons. *Neuron* 15: 1065– 

Sutor, B. and Hablitz, J. J. EPSPs in rat neocortical neurons in vitro. I. 
Electrophysiological evidence for two distinct EPSPs. *J. Neurophysiol.* 

Sutor, B., Jordan, W., and Zieglausberger, W. Evidence for a magne-
sium-insensitive membrane resistance increasing during NMDA-induced 
de polarizations in rat neocortical neurons in vitro. *Neurosci. Lett.* 75: 

Thangnipon, W., Takt, T., Brodal, P., and Storm-Mathisen, J. The 
corticopontine projection: anatomy-induced loss of high-affinity L-gluta-
mate and d-aspartate uptake, but not of γ-aminobutyrate uptake, gluta-
mate decarboxylase or choline acetyltransferase, in the pontine nuclei. 

Thier, P. and Koehler, W. Morphology, number and distribution of puta-
tive GABAergic neurons in the basilar pontine gray of the monkey. *J. 

Thomson, A. M., Deuchars, J., and West, D. C. Large, deep layer pyra-
mid-pyramid single axon EPSPs in slices of rat motor cortex display 
paired pulse and frequency-dependent depression, mediated presynapti-

Thomson, A. M., Deuchars, J., and West, D. C. Single axon excitatory 
postsynaptic potentials in neocortical interneurons exhibit pronounced 

Tsukahara, N. and Bando, T. Red nuclear and interoposite nuclear excita-

Wang, X. F. and Daw, N. W. Metabotropic glutamate receptors potentiate 
responses to NMDA and AMPA from layer V cells in rat visual cortex. 

Wang, Z. and McCormick, D. A. Control of firing mode of corticofetal 
corticopontine layer V burst-generating neurons by norepinephrine, 

Watt, C. B. and Mihailoff, G. A. The cerebellopontine system in the rat. 

Wiesendanger, R. and Wiesendanger, M. The corticopontine system in the 