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Locally evoked potentials in slices of the rat nucleus accumbens: NMDA and non-NMDA receptor mediated components and modulation by GABA

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In a slice preparation of the rat nucleus accumbens (Acb), local electrical stimulation elicited a field potential composed of two negative peaks, followed by a positive wave. The early negative peak was identified as a non-synaptic compound action potential, the late negative peak as a monosynaptic population spike (PS) and the positive wave as a mixture of an excitatory and an inhibitory postsynaptic potential (PSP). Both the PS and the PSP exhibited a marked degree of paired-pulse facilitation. The quisqualate/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 2 μ M) and the broadly acting glutamate receptor antagonist kynurenic acid (300 μ M) reversibly abolished or reduced both the PS and PSP. In contrast, nicotinic, muscarinic and *N*-methyl-D-aspartate (NMDA) receptor antagonists had no suppressive action. Washout of Mg^{2+} from the superfusion medium reversibly enhanced and prolonged the PSP and this effect was blocked by the NMDA receptor antagonist D(-)-2-amino-5-phosphonopentanoic acid (D-AP-5). The γ -aminobutyric acid antagonist picrotoxin (60 μ M) enhanced the PS and induced secondary spikes which were superimposed on a prolonged PSP. Most of this prolongation was abolished by D-AP-5. It is concluded that locally evoked synaptic responses in the Acb are mediated by glutamate or aspartate, and that NMDA receptor mediated activity evoked by low-frequency stimulation is substantial in Mg^{2+} -free medium or during reduced GABA_A receptor activity, but not under normal conditions.

INTRODUCTION

The distinction between the dorsal striatum, largely comprising the caudate-putamen complex, and the ventral striatum is generally accepted^{20,24,25}. The ventral striatum is constituted of the nucleus accumbens (Acb), the striatal elements of the olfactory tubercle and the most ventromedial parts of the caudate-putamen complex^{19,24,25}. The ventral striatum forms an important part of the so-called 'limbic striatum' as described by Kelley et al.³⁴.

Anatomical tracing studies have shown that the inputs and outputs of the dorsal and ventral striatum are organized in a parallel fashion^{20,24,25}. While the dorsal striatum receives its main input from the entire neocortex, the intralaminar thalamic nuclei and substantia nigra pars compacta, and sends a large output to the globus pallidus and substantia nigra pars reticulata, the ventral striatum is innervated by the subiculum, amygdala, prefrontal cortex, midline thalamic nuclei and ventral tegmental area (VTA) and projects to the ventral

pallidum, substantia nigra and VTA^{16,18,24,48,52,63}. Most input fibers from the subiculum and amygdala probably terminate on medium-sized neurons in the Acb¹¹ that are most likely GABAergic and peptidergic^{3,24}. Projection neurons in the Acb are thought to be mainly GABAergic and peptidergic^{21,31,62,63}.

Behavioral, anatomical and physiological studies have supported the notion that the Acb functions as an interface between the limbic system and structures mediating motor behavior^{16,25,37,45}. It has been suggested that the Acb is involved in several motor disorders, in schizophrenia and in drug addiction^{29,35,55–58}.

For a better understanding of the role of the Acb in normal and pathological behavior, it is necessary to unravel the intrinsic circuitry of the Acb and the functions of the transmitters involved. The role of the glutamatergic system has been little explored, although the inputs mentioned above, except those arising in the VTA, are probably glutamatergic according to transmitter-specific retrograde tracing^{6,14} and biochemical studies⁶⁴. Recently, Uchimura et al.⁶⁰ reported that fast

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excitatory potentials evoked by local electrical stimulation in a slice preparation of the Acb are mediated by glutamate.

Biochemical studies have shown a relatively high level of *N*-methyl-D-aspartate (NMDA) binding sites in the Acb⁴⁶. The occurrence of NMDA receptor activity in this brain area is of special interest since this receptor mediates induction of long-term potentiation in the hippocampus^{7,23,40}. As yet it is unknown whether it is involved in synaptic plasticity in the Acb as well. In the hippocampus, substantial NMDA receptor activity only occurs during high-frequency stimulation or during low-frequency stimulation in nominally Mg^{2+} -free medium^{27,39}. However, in slice preparations of the visual and entorhinal cortex, as well as subthalamic area, NMDA receptors markedly contribute to synaptic responses elicited by low-frequency stimulation and in the presence of Mg^{2+} (refs. 32, 33, 47). For the Acb it was suggested that NMDA receptors mediate the major part of the excitatory postsynaptic potential (EPSP) evoked by local electrical stimulation, but the results are somewhat ambiguous⁶⁰.

Whereas the pallidal and nigral areas probably receive a strong GABAergic input from the Acb, GABA may also act within the Acb itself. Projection cells have been shown to possess axon collaterals which terminate in the Acb. Indeed, results obtained by Chang and Kitai⁵ and Uchimura et al.⁶⁰ indicate that the fast EPSP elicited by local stimulation is followed by an IPSP mediated by GABA_A receptors. In this study, we specifically tried to determine whether GABA is able to counteract both NMDA- and non-NMDA receptor mediated activity.

We addressed these questions by investigating field potentials evoked by local electrical stimulation in slices of the Acb. Spike activity and postsynaptic potentials were recorded and analyzed using pharmacological tools to manipulate the glutamatergic and GABAergic system. In addition, we tested whether the cholinergic transmitter system contributes to the locally evoked potential⁴⁴.

MATERIALS AND METHODS

Slices were prepared from male Wistar albino rats (120–200 g) that had been anesthetized with ether. After decapitation, the brain was carefully removed from the skull and cooled in Ringer solution at 3–7 °C for 1–2 min. The lateral parts of both hemispheres were removed to leave the medial 3–4 mm of each hemisphere intact. After the trimmed hemispheres had been separated by a sagittal cut, the dorsal part of the cerebrum was removed by a horizontal cut about 1.0 mm dorsal to the decussation of the anterior commissure (AC). The ventral striatum was further trimmed by a horizontal cut just above the olfactory tubercle. Slices were obtained either by using a Sorvall tissue chopper (400 μ m thick) or by using a set of parallel-mounted razor blades (500 μ m thick)⁴⁰. In both procedures, the basal forebrain was cut in the transverse plane.

The slices were transferred to the recording chamber, placed

between a nylon mesh and a plastic net and completely submerged. They were continuously superfused (1–2 ml/min) with oxygenated (95% O₂, 5% CO₂) Ringer solution (33–35 °C, pH 7.3) of the following composition (in mM): NaCl 119, KCl 3.5, MgSO₄ 1.3, CaCl₂ 2.5, NaH₂PO₄ 1.0, NaHCO₃ 26.2, glucose 11.0. The slices were allowed 1 h rest prior to recording. They were kept in good condition for at least 10 h, even after long periods of low-frequency (0.15-Hz) stimulation. Bipolar, biphasic rectangular current pulses (40–550 μ A, generated by a Grass S-88 stimulator) of 0.2-ms duration were applied to the slice through two 60- μ m-thick stainless-steel electrodes, insulated except at the tip and separated by 100–200 μ m. The recording electrodes were glass micropipettes filled with 3 M NaCl (4–10 M Ω). Field potentials and unit activity were amplified and displayed on a digital Nicolet 3091 oscilloscope. In addition, they were sampled at 10.0 or 20.0 kHz, averaged ($n = 8$) on line using a Motorola Exorset microcomputer and stored on diskette for further analysis. Unit activity was usually sampled at 20 kHz and not averaged.

Stock solutions of tetrodotoxin (TTX), kynurenic acid, picrotoxin (PTX), γ -D-glutamylglycine (γ DGG), atropine (all from Sigma) and D-AP-5 (*Tocris neuramin*) were made up in distilled water. Pentobarbital (Onderlinge Pharmaceutische Groothandel, Netherlands) was solved in an alkaline saline solution, CNQX (*Tocris neuramin*) in dimethylsulfoxide. The latter drug was diluted 10⁴ or 2 $\times$ 10⁴ times for preparing test solutions. Dimethylsulfoxide alone had no effect on field potentials (4 slices tested). D-Tubocurarine (Asta-Werke) and pyridostigmine bromide (Hoffman-LaRoche) were available in dissolved form. The pH of the Ringer solutions was checked at least once for each drug added and was found to be 7.3–7.4.

Quantification and statistics

The threshold current needed for evoking an N2 component (Fig. 2A) was set at 0%; the minimal current intensity evoking a maximal N2-amplitude was set at 100%. Amplitudes of field potential components were measured with respect to the baseline preceding the stimulus. Statistical evaluation of pharmacological effects and paired-pulse facilitation of field potentials was done using Wilcoxon's matched-pairs signed-rank test. In other cases Student's *t*-test was used. Values between brackets denote mean $\pm$ S.E.M. unless noted otherwise.

RESULTS

Locally evoked field potentials and unit activity

In Fig. 1 an outline of a typical slice comprising the Acb and its border structures is sketched. On visual inspection of living slices, the borders of the Acb were recognized as follows. The medial border was clearly demarcated from the vertical limb of the diagonal band of Broca, the ventral taenia tecta and the medial and lateral septum by the zonula limitans²⁶. Dorsally, the Acb was distinguished from the caudate-putamen by the absence of fascicles of the internal capsule. The ventral boundaries of the Acb were formed by the olfactory radiations in the deep layers of the olfactory tubercle. The shape and position of the lateral ventricle, the septum, the corpus callosum and the AC were clear markers of the anterior–posterior level of the slice surface with respect to bregma⁵¹. The slices used were taken from about 1.0 and 2.5 mm anterior to bregma.

Electrical stimulation within the Acb evoked a com-

plex field potential, always consisting of an early negative peak (N1; mean peak latency: 1.45 ± 0.45 ms; $n = 60$) and a late negative peak (N2; mean peak latency: 4.0 ± 0.1 ms; $n = 65$; onset range 1.9–3.4 ms). N1 was usually followed by a small positive component, P1 (Fig. 2A), N2 by a relatively long-lasting positive wave, P2. This field potential could be recorded in all subregions of the Acb, although P2 was most clearly seen in the region surrounding the AC and in other border regions of the Acb. The position of the stimulation electrode did not matter in this regard. In 8 slices the position of the recording pipette was varied with respect to the AC while the stimulation electrode remained at the same location. In all but one slice it was shown that the amplitude of the P2 component increased towards the border of the AC but within the AC decreased to zero. These variations in P2 amplitude did not correlate to changes in the amplitude of N2. Across different experiments maximal N2 and P2 amplitudes were found at a distance of 0.2–1.0 mm from the stimulation site.

N1 and N2 usually appeared at lower stimulation intensities ($109 \pm 11 \mu\text{A}$; $n = 18$) than P2, but saturated at about equal intensities as P2 ($412 \pm 20 \mu\text{A}$). When shifting intensity from 10–20% to 90–100%, the peak latency of N2 decreased by 1.2 ± 0.2 ms ($n = 10$) whereas the peak latency of N1 did not change significantly (mean decrease 0.1 ± 0.1 ms; $n = 7$). The shift in the onset

latency of N2 (0.5 ± 0.1 ms; $n = 10$) was less than in the peak latency.

Several tests were performed to determine which components of the field potential are mediated by synaptic processes: (1) in 6 slices, a high-frequency (100-Hz) train of 10 stimulation pulses was applied to the slice. In all cases, N1 and P1 were observed to follow the stimuli faithfully, while N2 and P2 did not. (2) As illustrated in Fig. 2A–C, superfusion of medium containing 0.2 mM Ca^{2+} and 8.0 mM Mg^{2+} reversibly abolished the N2 and P2 component, while N1 and P1 were left intact ($n = 4$). From Fig. 2C and D it is evident that 0.5 μM TTX abolished all components of the evoked potential ($n = 4$); only an early, small positive component was left, which was presumably a stimulus artifact.

A conspicuous finding was that locally evoked field potentials showed a pronounced degree of paired-pulse facilitation. In Fig. 3A a typical example of paired-pulse facilitation is shown. Both N2 and P2 were strongly facilitated while N1 and P1 were not. At 80–100% stimulation intensity and 20 ms interstimulus interval, the N2 amplitude of the test response was 2.1 ± 0.3 ($n = 20$) times larger than that of the conditioning response. Likewise, P2 was enhanced by a factor of 2.1 ± 0.4 ($n = 8$) while N1 was not facilitated (1.05 ± 0.04 ; $n = 20$). At 20–30% stimulation intensity the ratio was 2.6 ± 0.4 ($n = 15$) for N2 and 2.9 ± 0.6 ($n = 6$) for P2. These mean values were significantly larger than at 80–100% stimulation intensity (Wilcoxon's matched-pairs signed-rank test; $P < 0.05$, $n = 6$ for P2 and $P < 0.01$, $n = 15$ for N2). The ratio for N1 (1.03 ± 0.03 ; $n = 13$) did not significantly differ from the ratio found at 80–100%. Paired-pulse inhibition was encountered in only two out

Fig. 1. Outline of a slice comprising the nucleus accumbens and its border structures. The anterior–posterior level of this slice according to the atlas of Paxinos and Watson⁵¹ is about 1.6 mm anterior to bregma. The width of the slice is 4.0 mm. The distinction between core and shell region can only be seen after immunohistochemical staining as described by Herkenham et al.²⁶ and Groenewegen et al.¹⁹. AC, anterior commissure; Acb-C, core region of the nucleus accumbens; Acb-S, shell region of the nucleus accumbens; CPu, caudate-putamen; LS, lateral septum; LV, lateral ventricle; OT, olfactory tubercle; VP, ventral pallidum; ZL, zonula limitans. Hatched areas represent white matter.

Fig. 2. The N1 and P1 components of the locally evoked field potential result from direct activation of axons and neurons, whereas N2 and P2 are synaptically mediated. In A, the components of the field potential are indicated. A–C: N2 and P2 were reversibly blocked by application of 0.2 mM Ca^{2+} and 8.0 mM Mg^{2+} , while N1 and P1 were not affected. C,D: application of 0.5 μM TTX abolished all components of the field potential. In these and all subsequent traces positive is upward.

of 20 cases tested and only at high-stimulus intensity (80–100%). The dependence of paired-pulse facilitation on interstimulus interval is shown in Fig. 3B. Paired-pulse facilitation reached a maximum at the 20-ms interval for N2, but sometimes it was still present at an interval of 200 ms ($n = 5$).

In order to relate the different components of the locally evoked potential to neuronal discharges, unit activity was studied. This type of activity was distinguished from population spikes by the methods outlined by Lemon and Prochazka³⁶. Spontaneous firing of single cells in the Acb slice was never encountered. In contrast, many units fired in response to local stimulation. Repetitive firing was encountered in only one case on a total number of 114 units.

Antidromically activated units were distinguished from orthodromically activated units by 3 criteria: (1) lack of jitter in unitary discharges; (2) high-frequency following (3–6 pulses, 100 or 150 Hz); (3) absence of changes in unitary discharge latency with varying stimulation intensity. In addition, units responding within 1.0 ms to stimulation can be identified as being antidromically activated³⁶. Of the 85 units tested, 53 were found to

respond orthodromically and 26 antidromically (6 units were found to respond sometimes orthodromically and sometimes antidromically). The amount of jitter in the orthodromic discharges was 0.35 ± 0.04 ms ($n = 51$). The antidromically activated neurons appeared to fire at a shorter latency (1.9 ± 0.2 ms; $n = 26$) than the orthodromically activated neurons (4.1 ± 0.1 ; $n = 53$; $P < 0.001$).

In Fig. 4 the number of unitary discharges is plotted against their latencies. For each unit, 1–4 randomly chosen discharges were incorporated in this histogram. The mean latency of the antidromic discharges was in the same range as the mean latency of the N1 peak of the field potential (see above). Likewise, the mean latency of the N2 peak almost coincided with the mean latency of the orthodromic discharges. These mean latencies were determined from the field potentials accompanying the spike events. Furthermore, the discharge latency and the latency of the field negativity were found to be significantly correlated (correlation coefficient 0.80, $n = 60$, $P < 0.001$). In the case of an antidromic unitary discharge N1 was chosen for the field negativity; N2 was chosen when an orthodromic unitary discharge was encountered. Finally, the orthodromic discharges were shown to be time-locked to the N2 peak. After recording discharges of single units at an intensity of 70–90%, the stimulation current was lowered to 10–30% and the latency shifts of both the N2 peak and the unit discharge were recorded. The correlation between these two latency shifts was

Fig. 3. The synaptic components of the field potential show a pronounced degree of paired-pulse facilitation. A: at an interval of 20 ms and at 50% stimulation intensity, the N2 and P2 component of the test response were about twice as large as those of the conditioning response. B: paired-pulse facilitation, expressed as the ratio of test response/conditioning response, was strongly dependent on the interstimulus interval. A maximum facilitation of N2 was reached when the two stimuli were 20 ms apart. At 5 ms the paired-pulse ratio was not calculated for the P2 component since the test stimulus overlapped the P2 component of the conditioning response at this interval. The responses in A and B were recorded at the same site.

Fig. 4. Identification of N1 as a non-synaptic compound action potential and N2 as a monosynaptic population spike. The number of unitary discharges is plotted as a function of their latency (bin width 0.25 ms). The total number of units was 114, each of which contributed 1–4 values of discharge latency to this histogram. The relative stimulation intensity was 70–90%. The mean peak latencies of the N1 and N2 component of the field potentials accompanying the unit discharges are represented by the two vertical lines drawn at full scale. The one-sided standard deviations (s.d.) are indicated by the two horizontal bars.

statistically significant (correlation coefficient 0.63, $n = 18$, $P < 0.01$).

The occurrence of paired-pulse facilitation of unitary discharges was tested at various interstimulus intervals. The stimulus intensity was adjusted just below the threshold necessary to evoke a unitary discharge in the conditioning response. Of 27 orthodromic units tested at a 20-ms interval, 21 discharged in response to the test stimulus, indicating facilitation, while 6 did not. The maximal interval at which facilitation was still detected was 125–250 ms (5 units).

The analysis of unit activity given here and the results presented in the following sections clearly support the notion that the N1 component of the locally evoked potential is a (non-synaptic) compound action potential (CAP), N2 a monosynaptic population spike (PS) and P2 a mixture of an EPSP and an IPSP, collectively called PSP (see Discussion). In the following sections, the conventional terminology will be used to denote these different components.

The transmitter system mediating the synaptic response

As mentioned above, the Acb is massively innervated by glutamatergic fibers originating in the subiculum, amygdala, prefrontal cortex and the midline thalamic nuclei. To investigate whether local stimulation is able to activate these fibers, slices were exposed to the quisqualate/kainate receptor antagonist CNQX and the broadly acting Glu/Asp receptor antagonists kynurenic acid and γ DGG. In these experiments, summarized in Table I, the amplitude of the CAP served to monitor possible non-specific changes in the response not due to effects on the synaptic response.

In Fig. 5A–C an example of the powerful antagonizing action of $2 \mu\text{M}$ CNQX is shown. It reversibly reduced the amplitudes of the PS and PSP to $8 \pm 3\%$ ($n = 9$; $P <$

Fig. 5. Quisqualate/kainate receptor antagonists reversibly abolish or reduce the synaptic response to local stimulation. A–C: $2 \mu\text{M}$ CNQX reversibly abolished the population spike and postsynaptic potential but did not affect the compound action potential. D–F: the population spike and postsynaptic potential were strongly reduced by $300 \mu\text{M}$ kynurenic acid. Washout time was about 45 min for CNQX and 15 min for kynurenic acid.

0.01) and $3 \pm 3\%$ ($n = 5$) of control values (washout values: $94 \pm 4\%$ and $93 \pm 6\%$) but did not affect the CAP ($107 \pm 5\%$ of control). Kynurenic acid ($300 \mu\text{M}$) had the same effects, although its antagonizing action was less powerful (Fig. 5D–F): the population spike was reduced to $29 \pm 6\%$ of control ($n = 10$; $P < 0.01$; washout: $100 \pm 4\%$) and the postsynaptic potential to $16 \pm 7\%$ of control ($n = 7$; $P < 0.02$; washout: $91 \pm 6\%$). The effects of both CNQX and kynurenic acid were observed in all subregions of Acb studied: at several locations along the anterior–posterior, dorsoventral and lateromedial axis in the core region of the Acb, and in the ventral and dorsomedial portion of the shell. The synaptic response was also decreased by application of $400 \mu\text{M}$ gDGG: the population spike was reduced to $72 \pm 4\%$ of control ($n = 6$; $P < 0.05$; washout: $94 \pm 4\%$) and the PSP component to $62 \pm 11\%$ of control ($n = 4$; washout: $91 \pm 9\%$).

Fig. 6. In Mg^{2+} -free medium, the postsynaptic potential is enhanced through activation of NMDA receptors. A,B: at 100% stimulation intensity, application of $0 \text{ mM Mg}^{2+}/50 \mu\text{M D-AP-5}$ solution did not affect the field potential significantly. The baseline is indicated by a dotted line. It must be noted that the peak latency of the population spike and postsynaptic potential were slightly shorter and their amplitudes slightly enhanced during application of $0 \text{ mM Mg}^{2+}/\text{D-AP-5}$ solution with respect to the control situation. C: in the same experiment, subsequent application of Mg^{2+} -free medium without D-AP-5 enhanced and prolonged the postsynaptic potential. Like in B, the compound action potential and population spikes were enhanced in Mg^{2+} -free solution. D: superimposed traces of A (control) and C (0 mM Mg^{2+} condition). The postsynaptic potential component was consistently enhanced after washout of Mg^{2+} . E: after reintroducing Mg^{2+} to the bathing medium, the response returned to control level.

To test whether NMDA receptors participate in mediating the synaptic response, Acb slices were superfused with Ringer solution containing 50 μ M D-AP-5 and stimulated at a low rate (0.15 Hz). A reduction of the postsynaptic response was not found: the amplitudes of the PS and PSP with respect to control level amounted to $102 \pm 2\%$ and $101 \pm 4\%$, respectively ($n = 4$).

In another series of experiments, the Acb slice was superfused with Mg^{2+} -free Ringer solution containing 50 μ M D-AP-5, and subsequently with Mg^{2+} -free solution (Fig. 6). D-AP-5 was co-administered with Mg^{2+} -free solution before Mg^{2+} -free solution alone was applied since the latter might induce a long-lasting enhancement of the response, like it does in the hippocampal slice preparation³⁹. When stimulation intensity was adjusted to 50%, superfusion of Mg^{2+} -free solution induced enhancements of CAP, PS and PSP to $115 \pm 6\%$ ($n = 7$; $P < 0.05$; washout: $100 \pm 6\%$), $139 \pm 12\%$ ($n = 7$; $P < 0.02$; washout: $104 \pm 17\%$) and $145 \pm 12\%$ ($n = 7$; $P < 0.05$; washout: $85 \pm 10\%$, not significant) of control levels, respectively. Superfusion of 0 mM Mg^{2+} /D-AP-5 solution elicited similar changes in all of these components: the CAP, PS and PSP increased to $116 \pm 5\%$ ($n = 7$; $P < 0.05$; washout: $98 \pm 2\%$), $149 \pm 12\%$ ($n = 7$; $P < 0.05$; washout: $106 \pm 3\%$) and $119 \pm 5\%$ ($n = 7$; $P < 0.02$; washout: $102 \pm 5\%$), respectively. Furthermore, during 0 mM Mg^{2+} superfusion the duration of the PSP was reversibly enhanced to $155 \pm 17\%$ of control level (Fig. 6; $n = 6$; $P < 0.05$; washout: $92 \pm 8\%$). This enhancement was strongly accentuated in the test response. During 0 mM Mg^{2+} and 50 μ M D-AP-5 superfusion the PSP component was not prolonged (mean duration of PSP relative to control response: $95 \pm 3\%$, $n = 6$, not significant).

Fig. 7. CNQX does not block NMDA-receptor mediated activity. A,B: application of 2 μ M CNQX blocked all synaptic components (cf. Fig. 5). C: in Mg^{2+} -free medium containing CNQX a small N2 and a prolonged P2 component emerged. D: application of 50 μ M D-AP-5 completely blocked the 0 mM Mg^{2+} -induced response. E: after returning to normal medium, the original response was restored. In all traces, the baseline is indicated by a dotted line.

We interpret the enhancement of the PSP component during 0 mM Mg^{2+} superfusion as being mediated by

TABLE I

Quantified effects of neuroactive substances on locally evoked potentials in the nucleus accumbens: means $\pm$ S.E.M. as % of control amplitudes

The locally evoked potential was measured at 70–90% stimulation intensity, except in experiments using 0 mM Mg^{2+} solution, 0 mM Mg^{2+} /D-AP-5 solution and picrotoxin, where the intensity was 50%. The amplitude was measured from peak to baseline. Statistical comparisons were made with respect to the control period preceding administration of the drug (15 min) using Wilcoxon's matched-pairs signed-rank test. n.s., not significant; (–), number insufficient for Wilcoxon's test ($n < 6$).

Substance	Number of experiments	Compound action potential	Population spike	Postsynaptic potential
CNQX	9	107 ± 5 n.s.	8 ± 3 ***	3 ± 3 (–)
Kynurenic acid	10	105 ± 2 n.s.	29 ± 6 ***	16 ± 7 **
γ DGG	6	96 ± 5 n.s.	72 ± 4 *	62 ± 11 (–)
D-AP-5	4	102 ± 4 (–)	102 ± 2 (–)	101 ± 4 (–)
D-AP-5 + 0 Mg^{2+}	7	116 ± 5 *	149 ± 12 *	119 ± 5 **
0 Mg^{2+}	7	115 ± 6 *	139 ± 12 **	145 ± 12 *
D-Tubocurarine	9	97 ± 6 n.s.	104 ± 4 n.s.	71 ± 8 (–)
Atropine	6	105 ± 3 n.s.	125 ± 10 n.s.	111 ± 6 (–)
PTX	9	103 ± 4 n.s.	179 ± 15 ***	63 ± 15 n.s.
Pentobarbital	6	104 ± 4 n.s.	60 ± 9 *	88 ± 9 (–)

* $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$.

NMDA receptors. This activity was not blocked by $2\ \mu\text{M}$ CNQX. In 3 slices it was shown that a small PS and a long-lasting positive wave could still be induced by Mg^{2+} washout during superfusion of $2\ \mu\text{M}$ CNQX. This response was completely blocked by $50\ \mu\text{M}$ D-AP-5. The positive component was of about the same amplitude as the additional component evoked by superfusion of Mg^{2+} -free solution in the absence of CNQX (Fig. 7).

Although the findings mentioned above provide evi-

dence for a glutamatergic mechanism of transmission, other transmitter systems may contribute to the locally evoked potential. Considering experiments in the dorsal striatum⁴⁴ it may be expected that acetylcholine is involved in mediating locally evoked potentials in the ventral striatum. If this is indeed the case, the reversible acetylcholinesterase inhibitor pyridostigmine should enhance or prolong the synaptic response. However, pyridostigmine ($50\ \mu\text{M}$) reversibly reduced the PS and PSP but did not affect the CAP ($n = 6$; results not shown).

The involvement of cholinergic transmission was further tested using $14\ \mu\text{M}$ D-tubocurarine and $100\ \mu\text{M}$ atropine. During D-tubocurarine application the amplitude of the CAP and PS amounted to $97 \pm 6\%$ and $104 \pm 4\%$ of control levels, respectively ($n = 9$, n.s.). The PSP amplitude, however, decreased to $71 \pm 8\%$ of control ($n = 4$). This effect was partially reversible by washing out for 30–60 min (recovery value: $79 \pm 6\%$; $n = 4$). Atropine slightly enhanced the PS and PSP to $125 \pm 10\%$ ($n = 6$, n.s.) and $111 \pm 6\%$ ($n = 4$) of control while the CAP remained close to control level ($105 \pm 3\%$, $n = 6$). These results were found at several recording sites throughout the Acb, i.e. in the dorsomedial and ventromedial parts of Acb and in the core, at a rostral as well as at a caudal level. Thus it appears that the role of ACh in the Acb is not to mediate the locally evoked response but is more likely to exert a depressant effect on synaptic responses.

Fig. 8. PicROTOXIN enhances the population spike and prolongs the postsynaptic potential; the latter effect is largely mediated by NMDA-receptors. A,B: at 50% stimulation intensity, the amplitude of the population spike was enhanced and a small N3 component appeared following application of $60\ \mu\text{M}$ picROTOXIN. C: these effects were reversible after 2–3 h wash. D: in a different experiment, picROTOXIN evoked two unitary discharges (indicated by arrows) which were superimposed on the population spike and N3, respectively. In the control situation, only one unitary discharge, coinciding with the PS, was found. Calibration bar is 0.5 mV for A–C and 1.0 mV for D. E,F: at 100% stimulation intensity and following paired-pulse stimulation, the picROTOXIN-induced enhancement of the postsynaptic potential and appearance of N3 were generally seen more clearly. G,H: these changes were largely and reversibly abolished by $50\ \mu\text{M}$ D-AP-5.

Fig. 9. Pentobarbital reduces the population spike and turns paired-pulse facilitation into paired-pulse inhibition. A,B: at 70% stimulation intensity, the population spike and postsynaptic potential of the conditioning response decreased during application of $100\ \mu\text{M}$ pentobarbital, while the population spike and postsynaptic potential of the test response were even more strongly reduced. C: these effects were largely reversible after washout of pentobarbital.

GABAergic modulation of glutamatergic transmission

The effects of blocking Cl^- channels coupled to GABA_A receptors by administration of $60 \mu\text{M}$ PTX were investigated at different stimulation intensities. At 50% intensity the amplitude of the PS increased to $179 \pm 15\%$ of control (Fig. 8A–C; $n = 9$; $P < 0.01$; washout: $124 \pm 13\%$, n.s.); the CAP remained close to control level ($103 \pm 4\%$; $n = 9$). In addition, a third, negative component (N3) appeared superimposed on the PSP. In most slices, the PSP apparently decreased in amplitude ($63 \pm 15\%$, $n = 7$, n.s.), due to the appearance of the N3 component. At 100% stimulation intensity and during paired-pulse stimulation it became obvious that the PSP was not suppressed, but masked by the N3 component. Fig. 8E,F shows that the PSP was in fact prolonged by PTX. The N3 component and the prolongation of the PSP were most clearly seen in the test response and thus exhibited paired-pulse facilitation. Under normal conditions orthodromic unitary discharges only occurred within the latency range of the PS, whereas during PTX discharges were also found within the latency range of N3 (Fig. 8D; 4 slices tested). This suggests that N3 is a secondary population spike. Threshold intensity was considerably lowered during PTX superfusion. All of these changes were reversible after prolonged washout.

By applying $50 \mu\text{M}$ D-AP-5 during superfusion of PTX we were able to show that a blockade of GABA_A receptor mediated activity allowed NMDA receptor mediated activity to emerge (Fig. 8E–H). At 80–100% stimulation intensity PTX induced an N3-component in the conditioning and test response, while the PSP was prolonged. The N3 component and the late phase of this prolonged PSP component were reversibly blocked or reduced by $50 \mu\text{M}$ D-AP-5 ($n = 4$).

Pentobarbital ($100 \mu\text{M}$), which enhances the efficacy of GABA receptors^{5,53}, had effects on both the conditioning response and paired-pulse facilitation. In the example shown in Fig. 9A–C a clear paired-pulse facilitation was present in the control situation. The PS of the test response was almost completely abolished during application of pentobarbital. In addition, application of pentobarbital reduced the PS and PSP of the conditioning response to $60 \pm 9\%$ ($n = 6$, $P < 0.05$; washout: $108 \pm 13\%$) and $88 \pm 9\%$ ($n = 5$; washout: $97 \pm 4\%$) of control values, respectively. In these experiments, the CAP remained close to control level ($104 \pm 4\%$; $n = 6$).

DISCUSSION

The field potential evoked by local stimulation in Acb slices has not been described before. Therefore, we will first discuss what type of neuronal responses the distinct components of the potential represent. Secondly, the

transmitter mechanism mediating the evoked potential will be discussed.

Analysis of locally evoked field potentials

The field potential can be subdivided into non-synaptic and synaptic components. The following arguments demonstrate that the N1 and P1 component are non-synaptic and N2 and P2 synaptic: (1) the amplitudes of N1 and P1 were invariant during high-frequency and paired-pulse stimulation. In contrast, the N2 and P2 did not follow frequencies of 100–150 Hz but exhibited a strong paired-pulse facilitation at intervals of 10–30 ms; (2) the peak latency of N1 did not depend on stimulation intensity whereas the peak latencies of N2 and P2 decreased with increasing intensity; (3) superfusion of 0.2 mM Ca^{2+} and 8.0 mM Mg^{2+} reversibly abolished N2 and P2 but not N1 and P1; (4) units discharging in the latency range covered by N1 were identified as being antidromically activated, whereas those discharging in the latency range covered by N2 responded in an orthodromic fashion; (5) N1 and P1 resisted manipulation by a number of neuroactive substances whereas N2 and P2 changed depending on the nature of the substance.

The complete block of field potentials by $0.5 \mu\text{M}$ TTX showed that all components are dependent on current flow through Na^+ channels. The close correspondence between the latencies of antidromic unitary discharges and the mean latency of N1 (Fig. 4) strongly suggests that this component consists of extracellular spikes not generated via synapses. Local stimulation in the Acb most probably generates action potentials in afferent and efferent fibers as well as directly in neuronal somata. Thus, N1 can be identified as a CAP.

Several observations clearly indicate that N2 is generated via a monosynaptic pathway: (1) the onset latency of N2 is in the range of 1.9–3.4 ms and decreases little with increasing stimulation intensity; (2) likewise, the latency shift in the N2 peak at increasing intensity is small and typical for monosynaptic responses; (3) the small amount of jitter encountered in orthodromic units characterizes monosynaptic responses¹¹. The latency and amplitude of P2 depend on stimulation intensity in a similar way as N2 does. Therefore, P2 is probably also the result of monosynaptic activation.

A strong temporal relationship between N2 and orthodromic unitary discharges was demonstrated. The significant correlation between the latencies of unitary discharges and the latencies of the accompanying field negativities (N1 or N2) underscores that these field negativities are constituted by unitary discharges. A time-locked relationship between the N2 component and the orthodromic unitary discharges was demonstrated by the significant correlation between the latency shifts of

orthodromic firing and of the N2 peak amplitude that were due to increasing stimulation intensity¹. These arguments strongly suggest that N2 is a PS. Its amplitude can be taken as a valid index for the number of cells firing synchronous in response to stimulation.

Whereas the CAP and PS could be elicited in all subregions of the Acb, P2 was most obvious in the border regions of the Acb. Although a definite demonstration has not been presented, we can advance arguments to support the interpretation that this component represents a mixture of an EPSP and an inhibitory postsynaptic potential (IPSP): (1) the amplitude of P2 strongly varied between different recording sites within the same slice. This spatial variability, which did not correlate to changes in the amplitude of N2, eliminates the possibility that P2 is a rebound effect of the PS. (2) P2 was abolished or reduced by application of antagonists of excitatory amino acid receptors. (3) Our experiments employing Mg^{2+} -free medium and D-AP-5 demonstrated that NMDA receptor mediated activity enhanced P2. (4) Block of inhibition mediated by GABA_A receptors enhanced and prolonged P2 and induced an N3 component, presumably a secondary population spike (Fig. 8D). This spike always occurred concomitantly with a prolonged P2. (5) Pentobarbital reduced the amplitude of P2, especially in the test response (Fig. 9). The two latter findings suggest that an IPSP is intermingled with an EPSP and may have, extracellularly, a polarity opposite to that of the EPSP. However, some caution is warranted as regards the specificity of pentobarbital, since it has also been reported to reduce excitatory amino-acid induced currents⁴¹. Intracellular recordings have shown that locally evoked synaptic potentials indeed consist of an EPSP followed by an IPSP, thus corroborating this interpretation (Pennartz et al., in preparation; refs. 5, 60).

A phenomenon not previously reported is the marked paired-pulse facilitation of locally evoked potentials and unitary discharges. It lasts about as long as in the hippocampus⁹ but longer than in slices of the dorsal striatum⁴³. Miller⁴² even reported a potent paired-pulse inhibition at high stimulus intensities in slices of the dorsal striatum. These findings may indicate a difference between the dorsal and ventral striatum in processing excitatory information.

Considering a number of studies reporting recurrent inhibition⁵⁰ and extensive collateralization of axons from medium-sized spiny neurons in the striatum⁴, the occurrence of a powerful facilitatory effect is surprising. We found that PTX enhanced paired-pulse facilitation of the PSP and of secondary spikes superimposed on the PSP. This finding suggests that, under normal conditions, GABA does have some inhibitory control over paired-pulse facilitation. Application of pentobarbital strongly

suppressed the test response. Since pentobarbital is generally assumed to enhance GABAergic inhibition, this finding suggests that under normal conditions GABAergic control over paired-pulse facilitation is not maximal.

The transmitter mechanism mediating the locally evoked potential and its modulation by GABA

In attempting to identify the transmitter system mediating the locally evoked synaptic response, the following results are of importance: (1) none of the drugs tested affected the CAP; therefore they are likely to specifically affect synaptic processes. Superfusion of Mg^{2+} -free medium, however, significantly enhanced the CAP, which may be explained by an enhancement of excitability due to the loss of membrane screening effects of divalent cations¹³; (2) 2 μM CNQX almost completely abolished the PS and PSP and 300 μM kynurenic acid and 400 μM γ DGG markedly reduced these components; (3) washout of Mg^{2+} resulted in an increased amplitude of the PS and PSP and prolongation of the PSP, but 50 μM D-AP-5 only abolished the prolongation and enhancement of the PSP; all of these effects were reversible; (4) 100 μM atropine was ineffective in suppressing the PS and PSP; 14 μM D-tubocurarine reduced the PSP but did not affect the PS; 50 μM pyridostigmine reversibly suppressed the PS and PSP.

The suppression of the PS and PSP by the quisqualate/kainate-receptor antagonist CNQX at micromolar concentrations demonstrates a powerful contribution of the Glu/Asp transmitter system to the locally evoked synaptic potential^{2,28,49}. This conclusion is corroborated by the antagonizing effects of kynurenic acid and γ DGG, which affect glutamatergic transmission in the hippocampus and the spinal cord in a similar concentration range as reported here^{7,10,15,54}. Subregions of the Acb appear to behave uniformly, since suppressive actions of these antagonists were found at many distinct locations in the Acb. This invariant pattern of activity may reflect the overall distribution of cortical and thalamic afferents, which, taken together, terminate in many different subregions of the Acb^{17,18,24,26,34,52}. The finding that D-tubocurarine and atropine did not antagonize the population spike is in agreement with our conclusion that the locally evoked synaptic response is mediated by glutamate or aspartate. This conclusion is further strengthened by the observation that neither haloperidol (5 μM), a dopamine antagonist, nor naloxone (1 μM), an opiate antagonist, affected the response (Pennartz et al., unpublished observations).

The presence of a NMDA-receptor mediated component in the PSP in Mg^{2+} -free medium confirms that local stimulation releases Glu/Asp from fibers terminating in

the Acb. The finding that application of D-AP-5 alone did not affect the synaptic response suggests that NMDA receptors do not significantly participate in synaptic transmission in normal superfusion medium. Uchimura et al.⁶⁰ reported that NMDA receptors mediate a major part of the locally evoked synaptic potential; however, the concentrations of D,L-APV (250 μ M) and D- α -aminoadipic acid (1 mM) they used may have had non-specific effects. Simultaneous application of CNQX and washout of Mg^{2+} revealed that CNQX, in a concentration of 2 μ M, does not block NMDA receptor mediated activity.

The changes in synaptic responses elicited by PTX and pentobarbital were essentially opposite: PTX reversibly enhanced the PS, prolonged the PSP and induced a secondary spike, which was most pronounced during paired-pulse stimulation and at saturating stimulation intensity. Pentobarbital strongly attenuated paired-pulse facilitation and reduced the amplitude of the PS and PSP in the conditioning response. These effects may be interpreted as indicating that under normal conditions local stimulation evokes GABA release and rapid activation of GABA_A receptors, which attenuate firing in Acb neurons. At present the origin of this GABA_A receptor mediated IPSP is unknown^{5,60}.

The finding that D-AP-5 blocked a large part of the PTX-induced enhancement and prolongation of the PSP, as well as the secondary spike, demonstrates that NMDA receptors are activated by local stimulation under the condition of reduced GABAergic inhibition. Apparently, shifting the balance between excitation and inhibition in favor of the former produces an amplification of excitation by activation of NMDA receptors. Previously,

Hablitz and Langmoen²² and Dingledine et al.¹² demonstrated a similar interaction in slices of the rat hippocampus. In the striatum, this phenomenon may prove to be important for studies of plasticity, development of myoclonic seizures and locomotor hyperactivity induced by picrotoxin injections in the Acb^{30,61}.

A final point to be discussed concerns the question to what extent the ventral striatum is physiologically similar to the dorsal striatum. Several studies of the dorsal striatum^{42,43,59} showed that local stimulation of slices evoked field potentials similar in shape and time course to the potentials reported here for the Acb. The first negative peak was also identified as a compound action potential not mediated via synapses, and the second peak as a monosynaptic PS⁴³. However, a clear PSP component was not reported in this structure. The PS, thought to arise from excitation of corticostriatal fibers, was reduced by low affinity glutamatergic antagonists^{8,38}. Atropine and D-tubocurarine were found to have no effect on this component⁵⁹. However, a blocking action of the nicotinic receptor antagonists D-tubocurarine and mecamylamine on the PS was reported by Misgeld et al.⁴⁴. Thus, in the dorsal striatum the PS may be mediated by a glutamatergic mechanism of transmission, but cholinergic mechanisms may also contribute. Application of D-AP-5, Mg^{2+} -free solution and quinoxalinediones to neuronal populations in the dorsal striatum may provide further insight into the physiological similarities between the dorsal and ventral striatum.

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REFERENCES

- Andersen, P., Bliss, T.V.P. and Skrede, K.K., Unit analysis of hippocampal population spikes, *Exp. Brain Res.*, 13 (1971) 208–221.
- Blake, J.F., Brown, M.W. and Collingridge, G.L., CNQX blocks acidic amino acid induced depolarizations and synaptic components mediated by non-NMDA receptors in rat hippocampal slices, *Neurosci. Lett.*, 89 (1988) 182–186.
- Bolam, J.P., Synapses of identified neurons in the neostriatum. In *Functions of the Basal Ganglia, Ciba Foundation Symposium 107*, Pitman, London, 1984, pp. 30–47.
- Chang, H.T. and Kitai, S.T., Projection neurons of the nucleus accumbens: an intracellular labeling study, *Brain Research*, 347 (1985) 112–116.
- Chang, H.T. and Kitai, S.T., Intracellular recordings from rat nucleus accumbens neurons in vitro, *Brain Research*, 366 (1986) 392–396.
- Christie, M.J., Summers, R.J., Stephenson, J.A., Cook, C.J. and Beart, P.M., Excitatory amino acid projections to the nucleus accumbens septi in the rat: a retrograde transport study utilizing D-[³H]aspartate and [³H]GABA, *Neuroscience*, 22 (1987) 425–439.
- Collingridge, G.L., Kehl, S.J. and McLennan, H., Excitatory amino-acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus, *J. Physiol.*, 334 (1983) 33–46.
- Cordingley, G.E. and Weight, F.F., Non-cholinergic synaptic excitation in neostriatum: pharmacological evidence for mediation by a glutamate-like transmitter, *Br. J. Pharmacol.*, 88 (1986) 847–856.
- Creager, R., Dunwiddie, T. and Lynch, G., Paired-pulse and frequency facilitation in the CA1-region of the in vitro rat hippocampus, *J. Physiol.*, 299 (1980) 409–424.
- Davies, J. and Watkins, J.C., Role of excitatory amino acid receptors in mono- and polysynaptic excitation in the cat spinal cord, *Exp. Brain Res.*, 49 (1983) 280–290.
- DeFrance, J.F., Marchand, J.F., Sikes, R.W., Chronister, R.B. and Hubbard, J.I., Characterization of fimbria input to nucleus accumbens, *J. Neurophysiol.*, 54 (1985) 1553–1567.
- Dingledine, R., Hynes, M.A. and King, G.L., Involvement of N-methyl-D-aspartate receptors in epileptiform bursting in the rat hippocampal slice, *J. Physiol.*, 380 (1986) 175–189.
- Frankenhaeuser, B. and Hodgkin, A.L., The action of calcium on the electrical properties of squid axons, *J. Physiol.*, 137 (1957) 218–244.
- Fuller, T.A., Russchen, F.T. and Price, J.L., Sources of presumptive glutamatergic/aspartergic afferents to the rat ventral striatopallidal region, *J. Comp. Neurol.*, 258 (1987) 317–338.
- Ganong, A.H., Lanthorn, T.H. and Cotman, C.W., Kynurenic

- acid inhibits synaptic and acidic amino acid-induced responses in the rat hippocampus and spinal cord, *Brain Research*, 273 (1983) 170–174.
- 16 Graybiel, A.M. and Ragsdale, C.W., Fiber connections of the basal ganglia. In M. Cuénot, G.W. Kreutzberg and F.E. Bloom (Eds.), *Development and Chemical Specificity of Neurons*, *Progress in Brain Research*, Vol. 51, Elsevier, Amsterdam, 1979, pp. 239–283.
 - 17 Groenewegen, H.J., Room, P., Witter, M.P. and Lohman, A.H.M., Cortical afferents of the nucleus accumbens in the cat, studied with anterograde and retrograde transport techniques, *Neuroscience*, 7 (1982) 977–995.
 - 18 Groenewegen, H.J., Vermeulen-Van der Zee, E., Te Kortschot, A. and Witter, M.P., Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of *Phaseolus vulgaris*-leucoagglutinin, *Neuroscience*, 23 (1987) 103–120.
 - 19 Groenewegen, H.J., Meredith, G.E., Berendse, H.W., Voorn, P. and Wolters, J.G., The compartmental organization of the ventral striatum in the rat. In A.R. Crossman and M.A. Sarnbrook (Eds.), *Neural Mechanisms in Disorders of Movement*, Libbey, London, 1989, pp. 45–54.
 - 20 Haber, S.N., Groenewegen, H.J., Grove, E.A. and Nauta, W.J.H., Efferent connections of the ventral pallidum: evidence of a dual striato-pallidofugal pathway, *J. Comp. Neurol.*, 235 (1985) 322–335.
 - 21 Haber, S.N. and Nauta, W.J.H., Ramifications of the globus pallidus in the rat as indicated by patterns of immunohistochemistry, *Neuroscience*, 9 (1983) 245–260.
 - 22 Hablitz, J.J. and Langmoen, I.A., *N*-Methyl-D-aspartate receptor antagonists reduce synaptic excitation in the hippocampus, *J. Neurosci.*, 6 (1986) 102–106.
 - 23 Harris, E.W., Ganong, A.H. and Cotman, C.W., Long-term potentiation in the hippocampus involves activation of *N*-methyl-D-aspartate receptors, *Brain Research*, 323 (1984) 132–137.
 - 24 Heimer, L., Alheid, G.F. and Zaborszky, L., Basal ganglia. In G. Paxinos (Ed.), *The Rat Nervous System*, Vol. 1, Academic, Sydney, 1985, pp. 37–86.
 - 25 Heimer, L. and Wilson, R.D., The subcortical projections of the allocortex: similarities in the neural associations of the hippocampus, the prepiriform cortex, and the neocortex. In M. Santini (Ed.), *Golgi Centennial Symposium*, Raven, New York, 1975, pp. 177–193.
 - 26 Herkenham, M., Moon Edley, S. and Stuart, J., Cell clusters in the nucleus accumbens of the rat, and the mosaic relationship of opiate receptors, acetylcholinesterase and subcortical afferent terminals, *Neuroscience*, 11 (1984) 561–593.
 - 27 Herron, C.E., Lester, R.A.J., Coan, E.J. and Collingridge, G.L., Frequency-dependent involvement of NMDA receptors in the hippocampus: a novel synaptic mechanism, *Nature*, 322 (1986) 265–267.
 - 28 Honoré, T., Davies, S.N., Drejer, J., Fletcher, E.J., Jacobsen, P., Lodge, D. and Nielsen, F.E., Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists, *Science*, 241 (1988) 701–703.
 - 29 Hornykiewicz, O., Psychopharmacological implications of dopamine and dopamine antagonists: a critical evaluation of current evidence, *Neuroscience*, 3 (1978) 773–783.
 - 30 Jenner, P., Leigh, P.N., Marsden, C.D. and Reavill, C., Cortical involvement in forepaw myoclonus induced by intra-striatal administration of picrotoxin to rats, *Br. J. Pharmacol.*, 72 (1980) 503P–504P.
 - 31 Jones, D.L. and Mogenson, G.J., Nucleus accumbens to globus pallidus GABA projection: electrophysiological and iontophoretic investigations, *Brain Research*, 188 (1980) 93–105.
 - 32 Jones, K.A. and Baughman, R.W., NMDA- and non-NMDA-receptor components of excitatory synaptic potentials recorded from cells in layer V of rat visual cortex, *J. Neurosci.*, 8 (1988) 3522–3534.
 - 33 Jones, R.S.G., Complex synaptic responses of entorhinal cortical cells in the rat to subicular stimulation in vitro: demonstration of an NMDA receptor-mediated component, *Neurosci. Lett.*, 81 (1987) 209–214.
 - 34 Kelley, A.E., Domesick, V.B. and Nauta, W.J.H., The amygdalostriatal projection in the rat — an anatomical study by anterograde and retrograde tracing methods, *Neuroscience*, 7 (1982) 615–630.
 - 35 Koob, G.F. and Bloom, F.E., Cellular and molecular mechanisms of drug dependence, *Science*, 242 (1988) 715–723.
 - 36 Lemon, R. and Prochazka, A., *Methods for Neuronal Recording in Conscious Animals*, Wiley, New York, 1984, pp. 89–112.
 - 37 Lopes da Silva, F.H., Arnolds, D.E.A.T. and Neijjt, H.C., A functional link between the limbic cortex and ventral striatum: physiology of the subiculum accumbens pathway, *Exp. Brain Res.*, 55 (1984) 205–214.
 - 38 Malenka, R.C. and Kocsis, J.C., Presynaptic actions of carbachol and adenosine on corticostriatal synaptic transmission studied in vitro, *J. Neurosci.*, 8 (1988) 3750–3756.
 - 39 Melchers, B.P.C. and Pennartz, C.M.A., Low magnesium-induced long-term potentiation in the rat dentate gyrus in vitro, *Neurosci. Res. Commun.*, 1 (1987) 113–118.
 - 40 Melchers, B.P.C., Pennartz, C.M.A., Wadman, W.J. and Lopes da Silva, F.H., Quantitative correlation between tetanus-induced decreases in extracellular calcium and LTP, *Brain Research*, 454 (1988) 1–10.
 - 41 Miljkovic, Z. and MacDonald, J.F., Voltage-dependent block of excitatory amino acid currents by pentobarbital, *Brain Research*, 376 (1986) 396–399.
 - 42 Miller, J.J., Characteristics of neuronal activity in striatal and limbic forebrain regions maintained in vitro. In G.A. Kerkut and H.V. Wheal (Eds.), *Electrophysiology of Isolated Mammalian CNS Preparations*, Academic, London, 1981, pp. 309–366.
 - 43 Misgeld, U., Okada, Y. and Hassler, R., Locally evoked potentials in slices of rat neostriatum: a tool for the investigation of intrinsic excitatory processes, *Exp. Brain Res.*, 34 (1979) 575–590.
 - 44 Misgeld, U., Weiler, M.H. and Bak, I.J., Intrinsic cholinergic excitation in the rat neostriatum: nicotinic and muscarinic receptors, *Exp. Brain Res.*, 39 (1980) 401–409.
 - 45 Mogenson, G.J., Jones, D.L. and Yim, C.Y., From motivation to action: functional interface between the limbic system and the motor system, *Prog. Neurobiol.*, 14 (1980) 69–97.
 - 46 Monaghan, D.T. and Cotman, C.W., Distribution of *N*-methyl-D-aspartate-sensitive L-[³H]glutamate-binding sites in rat brain, *J. Neurosci.*, 5 (1985) 2909–2919.
 - 47 Nakanishi, H., Kita, H. and Kitai, S.T., An *N*-methyl-D-aspartate receptor mediated excitatory postsynaptic potential evoked in subthalamic neurons in an in vitro slice preparation of the rat, *Neurosci. Lett.*, 95 (1988) 130–136.
 - 48 Nauta, W.J.H., Smith, G.P., Faull, R.L.M. and Domesick, V.B., Efferent connections and nigral afferents of the nucleus accumbens septi in the rat, *Neuroscience*, 3 (1978) 385–401.
 - 49 Neuman, R.S., Ben-Ari, Y., Gho, M. and Cherubini, E., Blockade of excitatory synaptic transmission by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in the hippocampus in vitro, *Neurosci. Lett.*, 92 (1988) 64–68.
 - 50 Park, M.R., Lighthall, J.W. and Kitai, S.T., Recurrent inhibition in the rat neostriatum, *Brain Research*, 194 (1980) 359–369.
 - 51 Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, Academic, New York, 1982.
 - 52 Phillipson, O.T. and Griffiths, A.C., The topographic order of inputs to nucleus accumbens in the rat, *Neuroscience*, 16 (1985) 275–296.
 - 53 Ransom, B.R. and Barker, J.L., Pentobarbital selectively enhances GABA-mediated post-synaptic inhibition in tissue cultured mouse spinal neurons, *Brain Research*, 114 (1976) 530–535.
 - 54 Robinson, M.B., Anderson, K.D. and Koerner, J.F., Kynurenic acid as an antagonist of hippocampal excitatory transmission,

- Brain Research*, 309 (1984) 119–126.
- 55 Scheel-Krüger, J. and Arnt, J., New aspects on the role of dopamine, acetylcholine, and GABA in the development of tardive dyskinesia. In Casey, Chase, Christen and Gerlach (Eds.), *Dyskinesia — Research and Treatment (Psychopharmacology Suppl. 2)*, Springer, Berlin, 1985.
 - 56 Seeman, P., Dopamine receptors and the dopamine hypothesis of schizophrenia, *Synapse*, 1 (1987) 133–152.
 - 57 Stevens, J.R., An anatomy of schizophrenia?, *Arch. Gen. Psychiatry*, 29 (1973) 177–189.
 - 58 Swerdlow, N.R. and Koob, G.F., Dopamine, schizophrenia, mania and depression: toward a unified hypothesis of cortico-striato-pallido-thalamic function, *Behav. Brain Sci.*, 10 (1987) 197–245.
 - 59 Takagi, M. and Yamamoto, C., Suppressing action of cholinergic agents on synaptic transmissions in the corpus striatum of rats, *Exp. Neurol.*, 62 (1978) 433–443.
 - 60 Uchimura, N., Higashi, H. and Nishi, S., Membrane properties and synaptic responses of the guinea pig nucleus accumbens neurons in vitro, *J. Neurophysiol.*, 61 (1989) 769–779.
 - 61 Wachtel, H. and Andén, N., Motor activity of rats following intracerebral injections of drugs influencing GABA mechanisms, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 302 (1978) 133–139.
 - 62 Walaas, I. and Fonnum, F., The distribution and origin of glutamate decarboxylase and choline acetyltransferase in ventral pallidum and other basal forebrain regions, *Brain Research*, 177 (1979) 325–336.
 - 63 Walaas, I. and Fonnum, F., Biochemical evidence for gamma-aminobutyrate containing fibres from the nucleus accumbens to the substantia nigra and ventral tegmental area in the rat, *Neuroscience*, 5 (1980) 63–72.
 - 64 Walaas, I. and Fonnum, F., Biochemical evidence for glutamate as a transmitter in hippocampal efferents to the basal forebrain and hypothalamus in the rat brain, *Neuroscience*, 5 (1980) 1691–1698.