

The nucleus accumbens (Acb) receives inputs from various limbic structures, namely the subiculum and other limbic cortical fields,\(^{10}\) the amygdala\(^ {11}\) and the limbic related thalamic nuclei.\(^{1}\) The Acb also receives inputs from the midbrain dopaminergic cell groups (i.e. ventral tegmental area, VTA). It projects to the substantia nigra pars reticulata and dopaminergic cell groups A8, A9 and A10 (VTA). It has been known for a long time that the Acb projects to the ventral pallidum\(^ {13}\) (VP) and subpallidal areas.\(^{19}\) The output fibres are mainly GABAergic and thus may exert an inhibitory influence on target neurons.

In order to understand how limbic influences are mediated through the Acb to more caudally lying areas, it is of importance to know how the limbic inputs affect Acb output neurons. Here we describe experiments carried out to clarify how inputs arising from the hippocampal formation affect the neuronal networks of the Acb in vivo. Yang and Mogenson\(^ {27}\) reported excitatory responses in a large number of units in the medial Acb following stimulation of the ventral subiculum, 37% of which could be identified as projection cells to either VP or subpallidal areas. Thus, 63% of the excitatory responses were recorded from unidentified cells. Inhibitory responses following subicular or fornix stimulation have also been reported.\(^ {26}\) However, the balance between excitatory and inhibitory action is, as yet, not well documented.

De France \textit{et al.}\(^ {5}\) investigated a possible interaction between successive responses using paired-pulse stimulation with intervals in the range of 10–40 ms. They showed that the response to the second, or test stimulus was suppressed at intervals below 30 ms. In a separate paper\(^ {1}\) these authors reported a suppressive effect of dopamine on the responses when the input fibres were stimulated once every 1–2 s. For stimulation with a rate of 6 pulses/s (intervals 167 ms) no such effect was observed.

These experimental findings led us to formulate the hypothesis that the transmission of signals through the neuronal networks of the Acb may take place in

\textbf{Abbreviations:}  Acb, nucleus accumbens; fo/fI, fornix–fimbria bundle; IS/SD, initial segment/somatodendritic; NMDA, N-methyl-D-aspartate; PSTH, peristimulus time histograms; Sub\(_{\text{caud}}\), subiculum, dorsal resp. ventral portion; VP, ventral or subcommissural pallidum; VTA, ventral tegmental area.
a frequency-dependent manner. Therefore, we studied the responses of the Acb, both evoked potentials and unit firing rates, to paired-pulse stimulation of the fornix and the subiculum systematically to a wide range of intervals. Parts of the results have been presented in abstract form.

EXPERIMENTAL PROCEDURES

Surgery

Male Wistar rats (Harlan, Zeist, The Netherlands) weighing 200–250 g were tracheotomized under halothane anaesthesia and additional local anaesthesia, in order to place a tracheal tube. This tube was connected to a respirator, and the animal was ventilated by a mixture of $O_2$ and $N_2O$ with 0.3–0.4% halothane throughout the experiment. Expired $CO_2$ was monitored continuously. Depth of anaesthesia was maintained at a level where a weak reflex of the eyelid to a drop of saline was present, and was controlled by checking whether the respiration followed the respirator pump. The animal was placed in a Kopf stereotaxic frame. The pressure points of the ear bars and of the mouth/nose clamp were infiltrated with xylocaine. The skin overlying the skull was incised and burr holes were made according to stereotaxic coordinates of the atlas of Pellegrino et al. An array of four parallel stimulation electrodes (stainless steel wires $\phi$ diameter 100 $\mu$m, insulated except at the tip) was aimed at the fornix–fimbria (fo/fi) fibre tract by way of a micromanipulator. A bundle of three or four stainless steel electrodes with 0.8–1.0 mm distance between the upper and lower tips, was used for recording from the dorsal subiculum (Sub). The placement of the fo/fi array was optimized by applying electrical stimulation with standard pulses (0.3–0.8 mA; 0.2 ms) to two of the four leads in the fornix while moving the array by small increments to the depth (Fig. 1A) where the maximal response was evoked in the Sub.

The electrodes were fixed to the skull using dental cement. Next, the Sub bundle was manipulated until the upper electrode displayed a negative-going wave following fo/fi stimulation, and the lower lead showed a rather sharp positive-going peak (Fig. 1B,C). This bundle was then also cemented to the skull. Similar bundles were placed in the ventral subiculum (Sub) and VP under electrophysiological control. The former were placed under an angle of 7° with the vertical axis, tip pointing laterally, the latter with an angle of 8.5° pointing medially. After all bundles were fixed and the responses verified once more, one or two glass microelectrodes, filled with 2% Pontamine Sky Blue in 0.5 M acetate buffer, pH 8.2 (resistance 15–30 MΩ), were lowered in coarse steps to the upper margin (approx. 5.5 mm under the surface of the neocortex) of the Acb. From this position the microelectrodes were moved using a fine, remotely controlled, hydraulic manipulator. The cortical tissue was covered with paraffin oil. During the recordings a Faraday cage was placed over the preparation.

Recording and data-acquisition

Microelectrodes were connected to the head stage(s) of voltage-follower(s) (WPI S7061A or M-4A amplifier). Sig-
nals were amplified and digitized by way of an interface (CED 1401) that was connected to an IBM-PC. Evoked potentials were sampled at a rate of 1000 samples/s, averaged (n = 16) and stored on hard-disk. In case action potentials were recorded, the unfiltered signals were sampled at 50 kHz, and individual sweeps with the spikes were stored. These signals were also band-pass filtered between 300 and 4000 Hz (Krohn-Hite, 18 dB/oct.). Spike events were singled out using an amplitude discriminator (Frederick Haer & Co.), the output of which was fed to a digital port of the CED interface. On-line, peristimulus time histograms (PSTHs) were constructed, usually consisting of 32 sweeps, and 1-ms time bins.

Standard experiments consisted of recording averaged field potentials evoked by fo/fi stimulation at different depths in steps of 250 μm, while unit activity was looked for throughout the whole penetration. In this way a number of units responding to fo/fi stimulation were detected. Besides, units firing spontaneously were also encountered. In order to test the responses to different inputs, the subiculum and VP were stimulated sequentially.

Following VP stimulation, we tested whether the unitary discharges fulfilled the criteria for antidromic activation according to Fuller and Schlag. Whenever action potentials occurred at a fixed latency, it was tested whether the cell followed reliably high frequency stimulation using paired-pulses with intervals <10 ms. Occasionally, the collision test was carried out using both VP and fo/fi stimulation.

**Histological processing**

At the end of the experiment, under deep anaesthesia, Pontamine Sky Blue was ejected from the electrode by passing current (approx. 5 μA for 20 min, electrode as cathode). Similarly, stainless steel electrodes were marked by passing three 0.4-s blocks of 1 mA anodal current. The animal was perfused transcardially with saline, followed by 5% glutaraldehyde in 0.05 M sodium acetate buffer (pH 4.0) with ferrocyanide. The brain was quickly removed and post-fixed for 1 h in the same solution. Then the brain was placed in Tris–NaCl buffer (pH 7.2) containing 30% sucrose and an anti-oxidant (1% sodium bisulphite). After at least one night frozen sections (thickness 40 μm) were cut on a microtome; these were incubated for immunohistochemical staining as described by Vosku et al. in order to facilitate the demarcation of the different subdivisions of the Acb. Antibodies against enkephalin and substance P were used routinely. At each level one slice was stained with Cresyl Violet.

**Off-line analysis**

The moments of occurrence of both the positive and negative maxima of the field potentials (see Results, Fig. 2) were determined off-line. In the case of paired-pulse stimulation the response to the second, or test pulse was influenced by the tail of the response to the first, or conditioning stimulus of the pair. Therefore, we used relative measures (peak–trough) in order to compare the amplitudes of the response to the test pulse with those of the response to the conditioning stimulus.

Characterization of the unit responses was carried out using the PSTHs: onset-latencies were defined by the time difference between the stimulus and the first bin showing a change in firing rate with respect to pre-stimulus baseline. Latency-variation by the width of the peak (the number of consecutive bins showing clearly increased, or decreased, firing). We defined the probability of responding (for non-bursting cells) as the ratio between the counts within these consecutive bins and the number of sweeps. To quantify the degree of paired-pulse facilitation we used the ratio of the number of spikes of the response to the test stimulus and that following the conditioning stimulus.

**RESULTS**

**Response to fornix–fimbria stimulation**

In general the responses in the Acb to electrical stimulation of the fo/fi bundle, along which the subicular outputs project to the Acb, consisted of two positive waves (P1 and P2), with peak-values at 10 (S.D. ± 1, n = 18) and 27 (S.D. ± 2) ms, respectively. In between these two waves a negative-going component (N1) could be distinguished (Fig. 2A,B). This initial complex was followed by a slow negative deflection (N7), that could last from 30 to over 100 ms. In most cases, as shown here, a positive-going deflection was superimposed on this N2-wave, but this component was less consistent than those described above. Finally, a long-lasting positive wave was present for several hundred milliseconds (Fig. 2A). This type of response could be recorded from the major part of the Acb. At the dorsal margins, the early positive waves reversed polarity, whereas this happened at the ventral border for the N2 component.

The subicular evoked potentials also showed an initial deflection with two positive peaks in 32% of the cases (Fig. 2C). It is of interest to mention that these responses had less steep slopes indicating that the incoming volley was more dispersed in time than in the case of fo/fi stimulation. In the other instances the two peaks could not be distinguished as shown in Fig. 2D; in these cases a small amplitude positive deflection with a duration of 20 40 ms was followed by a shallow negative component. It appears that both fo/fi and subicular stimulation can evoke excitatory potentials that have similar components but that those of the former are more sharply defined. Therefore, in the following main emphasis will be given to the results of fo/fi stimulation.

**Paired-pulse facilitation of evoked potentials**

A new effect found in these experiments was a marked paired-pulse facilitation of the excitatory potentials. Applying two identical electrical pulses at an interval of 100 ms gave rise to an augmentation of the response to the second or test stimulus. At low stimulus intensities, where a response to the first or conditioning stimulus could hardly be seen, a response to the test stimulus clearly emerged (Fig. 2B, trace 0.3 mA). The amplitudes P1–N1, N1–P2 and P2–N2 were determined, and plotted as a function of stimulus intensity (Fig. 2E,F,G). As can be seen, for strong stimulation the amplitude of the response to the conditioning stimulus was always smaller than that following the test stimulus, at least for the P2 and N2 components.

We investigated the time span at which such an enhancement was present by applying double pulse stimulation at different intervals. In all these measurements the same stimulus intensity, that was close to saturation, was chosen. Quantification of the amplitudes as described above was carried out for the first
and second responses. The N1–P2 and P2–N2 parameters showed strongest facilitation within approximately similar ranges of stimulus intervals. Therefore, only examples of [N1–P2] (Fig. 2) indicate absolute values) are shown as a function of interval in Fig. 3. It can be seen that a depression occurred around 40 ms, and that significant increases in amplitude were found between 60 and almost 300 ms, with maximal facilitation (ratio test/conditioning response 1.5) between 100 and 200 ms. Statistical analyses carried out in the same way as described in the legend of Fig. 3 on four other experiments showed that a depression was present at intervals of 20–40 ms, and that facilitation was present between 80 and at least 200 ms. At the interval of 100 ms we determined the [N1–P2] values for 10 additional experiments. The mean of the test/conditioning ratios was 1.7 (S.D. ± 0.4).

It should be mentioned that in case of subiculum stimulation in general facilitation was also found with an optimum between 100 and 200 ms (cf. Fig. 2C,D).

Field potentials following pallidal stimulation

Following stimulation of the VP, the evoked field potential can be characterized by a positive peak at 12 ms (S.D. ± 3, n = 10) followed by a negative deflection, peaking at 43 ms (S.D. ± 8). These responses did not show paired-pulse facilitation, and the positive component was unaffected by double pulse stimulation at intervals of 15–20 ms. This indicates that the component at about 12 ms represents antidromic activation of the population of
Fig. 3. Paired-pulse facilitation of field potentials. Amplitude [N1-P2] as a function of interval. At t = 0 the mean (± S.D.) of nine independent averaged first responses (control) is depicted (each response represents an average of 32 sweeps). At each interval the [N1-P2] parameter was statistically compared with the mean value of the control, using the sample t-test with eight degrees of freedom.24

the Acb output neurons. In order to find out to what extent the same neuronal populations as those responsible for the fo/fi evoked field potential are involved in the antidromic activation, a combined stimulus paradigm was used in a few cases. This consisted in stimulation of the VP at different intervals after or before applying a pulse to the fo/fi. It was found that the VP responses were strongly reduced when the stimulation of the VP followed fo/fi stimulation by 20–25 ms. It is of interest to mention that this interaction was found when recording from the lateral aspects of the core (see below) of the Acb.

Analysis of unit activity

In order to unravel the mechanisms responsible for the generation of field potentials and the paired-pulse facilitation we carried out extracellular recordings at the level of single units.

Since maximal facilitation was present in the field potentials at intervals around 100 ms, the standard procedure was to use paired-pulse stimulation at this interval. In 27 rats stable recordings were made of 60 units, the large majority of which represented single cell registrations. These concerned spontaneously active as well as silent cells. The spontaneously active cells present firing rates which could reach values of 100 spikes/s. The majority of cells fired with a single spike, but complex bursting cells were also occasionally encountered. Although we cannot infer the effects of electrical stimulation on the membrane potential of the single cells from the extracellular recordings, we will call responses characterized by an increase in firing excitatory, whereas those with a decrease of firing will be referred to as inhibitory responses. For the sake of completeness it should be stressed that the latter could be obtained from spontaneously active units only.

Unit responses following fornix–fimbria stimulation

Of the 60 units, 37 exhibited changes in firing rate following fo/fi stimulation. Of these 37, 25 were spontaneously active in rest and 12 were silent; 26 (70%) had excitatory responses, 11 inhibitory (Table 1).

An illustration of the response of a single cell is shown by Fig. 4A. In this case the onset latency, as revealed in the PSTH (Fig. 4B), was 25 ms. The distribution of the onset-latencies for all excitatory responses appeared to have more than one peak (Fig. 5), one around 10 ms, and another around 24–26 ms. The responses could also be distinguished according to their latency variation. Those with onset-latencies around 10 ms showed peaks in the PSTHs that had a mean width of 4 ms (S.D. ± 2, n = 11), whereas the duration of units with longer latencies ranged from 7 to 48 ms (cf. Fig. 4B). Four units displayed responses consisting of two spikes in register with each of the two latency clusters.

Action potentials were either monophasic positive (Fig. 4A) or biphasic (positivity first) in the majority of cases and had durations of 0.5–1.2 ms. Initial segment/somatodendritic (IS/SD) breaks were seen in spikes of both latency clusters.

The shortest onset-latencies of the inhibitory responses measured were around 12 ms, but these were more variable and could in a few cases reach more than 30 ms. For the stimulus intensities we normally used, the duration of the suppressed activity ranged from 40 to 174 ms. Therefore, the period of inhibition largely coincided with the N2 component of the evoked potential. In a substantial number of cases, the moment at which the firing rate of a cell returned

<table>
<thead>
<tr>
<th>Stimulation site</th>
<th>Number of cells tested</th>
<th>Number of cells responding</th>
<th>Inhibitory responses*</th>
<th>Excitatory responses*</th>
</tr>
</thead>
<tbody>
<tr>
<td>fo/fi</td>
<td>60</td>
<td>37 (62%)</td>
<td>11 (BF+)</td>
<td>14 (BF+)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12 (BF-)</td>
<td>6 (BF+)</td>
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<td></td>
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<td></td>
<td></td>
<td>1 (BF-)</td>
</tr>
<tr>
<td>Subv</td>
<td>20</td>
<td>11 (55%)</td>
<td>4 (BF+)</td>
<td>13 (A)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7 (S)</td>
</tr>
<tr>
<td>VP†</td>
<td>28</td>
<td>25 (89%)</td>
<td>5 (BF+)</td>
<td></td>
</tr>
</tbody>
</table>

*Background firing: present (BF+) or absent (BF−).
†Excitatory responses: (A)ntidromic or (S)ynaptic.
Fig. 4. Examples of unit responses. (A) Single sweep showing the response of a non-spontaneously active single cell, responding to fo/fi stimulation (artefact at 10 ms from beginning of sweep) with one action potential (sampling rate 50 kHz). (B) PSTH constructed using 32 cell responses as shown in A. (C) Averaged field potential ($n = 16$) corresponding to the same derivation as in A and B. Calibration A and C = 0.5 mV, 10 ms.

to baseline or showed a phasic overshoot, occurred at the transition of the N2 wave to the late positive component (cf. Fig. 2A,B) of the evoked potential.

Inhibitory responses appeared to be less dramatically affected by double pulse stimulation as was the case for the excitatory responses (see below).

Responses to subicular stimulation were obtained from 10 spontaneously active cells and one silent neuron. The number of excitatory and inhibitory responses are shown in Table 1. The latencies of excitatory responses were found predominantly in the range of 26-36 ms (Fig. 5).

All 26 units that presented excitatory responses to fo/fi stimulation and the seven units that were activated following subicular stimulation showed, regardless of latency, facilitation at intervals of 100 ms. We found that the ratio between the number of spikes of the response to the test pulse with respect to that (for the same time bins) of the response to the conditioning pulse was for the 26 fo/fi responses $4.0 \pm 0.6$. In several cases the facilitation was investigated for different intervals. It was shown that paired-
Paired-pulse facilitation in the nucleus accumbens

Fig. 6. Paired-pulse facilitation of unit responses. (A) Example of a PSTH constructed of 32 sweeps in which double pulse stimulation was applied. Note the increased probability of firing following the test pulse in comparison with that after the conditioning stimulus. (B) Probability of responding (integrated over seven bins) for the first (mean + S.D. at t = 0) and second responses as a function of interval. For statistical analysis see legend of Fig. 3. Note the increased probabilities in the range of 100–500 ms intervals.

The excitatory response was followed by a period of inhibition only in one case following fo/fi stimulation, and in another case following Sub3 stimulation. Of the 28 units tested 25 showed a response to stimulation of the ventral pallidal region. Twenty of
these had excitatory responses, 13 of which could be qualified as antidromic. These responses had short latencies although they varied considerably among units (mean 7.4, S.D. ± 4.3 ms). We used paired-pulse testing at intervals of approximately 10 ms for antidromic characterization. Under these stimulation conditions the latency of the second response was consistently smaller than that of the first (mean 6.9, S.D. ± 4.3 ms).

The remaining seven excitatory and five inhibitory responses (Table 1) were activated orthodromically. In contrast with the fo/fi evoked responses these excitatory responses showed only a weak facilitation (factor between 1 and 1.5) when tested at intervals of 100 ms; in one case the excitatory response was followed by inhibition. In three units an initial inhibitory response was followed by an excitatory burst, that displayed strong paired-pulse facilitation (ratio 2.0–3.8).

Interactions of the limbic inputs and pallidal outputs

Nine single units responded to stimulation of both fo/fi and VP. Four of these nine units gave excitatory responses to fo/fi stimulation, three of which were antidromically activated by pallidal stimulation. These cells thus may be identified as projection cells. In one case we could successfully demonstrate a collision of the fo/fi evoked spike with the antidromically induced spike. In this case the response elicited by fo/fi stimulation had a short latency (9.5 ms). This means that this projection cell received a monosynaptic input.

In another case a unit responded orthodromically to stimulation of both pallidal and fo/fi inputs. Five units showed an inhibition to stimulation of both areas. A possible interpretation is that in these cases the fo/fi stimulation activated orthodromically a GABAergic output neuron, that gave off a collateral forming a disynaptic pathway to the target cells, whereas the stimulation of the pallidum activated the same collateral thus activating a monosynaptic GABAergic pathway.

Regional distribution

On the basis of the microscopical sections stained for substance P, the Acb could be subdivided in the core and shell region. In relation to this partition, the recording sites were reconstructed as shown in Fig. 7. It should be noted that the number of recorded sites were about the same in the core and the shell. Excitatory responses following fo/fi stimulation were found in both the core and the shell region, and displayed paired-pulse facilitation in all cases. Inhibitory responses were found in the shell only, whereas cells activated antidromically following VP stimulation were found only in the core. The clustering of the projection cells in the core region presumably reflects the topographical distribution of ventral striatopallidal pathways.

DISCUSSION

Basic electrophysiology

The waveform of the field potentials elicited in the Acb by stimulation of fo/fi in the rat consisted of two early positive components peaking at 10 (P1) and 27 (P2) ms. Similar, but also different results have been reported in the literature for the rat and other species. In cat, a positive wave, compatible with the P1 wave presented here, was found at approximately 10 ms. DeFrance et al. described in the rabbit a negative field potential, with peak latency at approximately 8 ms, but in the ventral portion of the Acb a positive component with a peak latency at 10 ms was present (see Fig. 7 in Ref. 5). In rat, field potentials with two negative-going deflections at 7 and 20 ms were reported, but these authors noted that the field profile appeared to differ depending on where, within the Acb, the recording was made. However, in our experience the waveform we described was consistently found inasmuch as the recording was from within the Acb. At the dorsal margins of the Acb, the early positive waves could be seen to reverse polarity.

The shape of the evoked responses to subiculum stimulation shown in Fig. 2D is in agreement with those reported in the cat. In the latter studies peak latencies were found between 25 and 30 ms, and the positive wave was accompanied by increases in extracellularly recorded unit activity. The complex subiculum responses as shown in Fig. 2C has, to the best of our knowledge, never been reported before.

In the present study we can conclude from the recordings of single unit activity that the field potentials are generated within the Acb. We may state that the latencies of excitatory responses (10 and 24–26 ms) cluster roughly around the two positive
components of the evoked potential. These results show a striking similarity with those reported by Hakan and Henriksen who found latencies in the rat around 7 and 20 ms following fimbria stimulation. A distribution of unit latencies with more than one peak following fo/fi stimulation has also been described in rabbit (around 8 and 26 ms) and in cat for both fo/fi (around 25 and 50 ms) and ventral subiculum stimulation (around 30 and 55 ms).

In the rat the latencies of excitatory responses recorded from the medial Acb following electrical stimulation of the ventral subiculum fell in the range of 6–20 ms. Holland and Soedjono reported latencies in the range of 18–30 ms following hippocampal stimulation. Differences in the latency values may have been due to differences in electrode positions. Furthermore, spontaneously active cells appear to have longer latencies than silent cells.

Implications for the circuitry

The presence of IS/SD breaks in the spikes indicate that the action potentials were generated postsynaptically in the neurons of the Acb. The collision of a fo/fi evoked spike with an antidromically travelling spike (from the VP) indicates that, at least in this case, these short latency spikes may be caused by monosynaptic activation. The PSTH peaks had a mean width of 4 ms. Most likely synaptic delays are responsible for this variation (jitter). Monosynaptic activation of Acb units by subicular stimulation was also found by Yang and Mogenson. It is of interest to mention that at the level of neuronal populations, we found a suppressive effect on the field potential evoked by VP stimulation when it was preceded by a fo/fi conditioning pulse by 20–25 ms. It is possible that this attenuation is due to numerous collisions of ortho- and antidromically travelling action potentials in the ventral striatopallidal pathways, but we cannot exclude that this effect may also be due to stimulation of inhibitory collaterals. We computed conduction velocities for the fo/fi fibres. These fell in the range of 0.5–0.6 m/s. This is much lower than those found in the literature (cat: 1.7 m/s, rabbit: 1.7–2.2 m/s). It should be mentioned, however, that the unit latencies were comparable with those reported for the rat (see above). For the excitatory responses with longer latencies (around 24–26 ms) we cannot give a definitive interpretation. One possibility is that slowly propagating fibres are involved, but the conduction velocities for the fibres responsible for the short latency excitatory responses were already rather low. Another possibility is that the longer latencies are the result of a di- or polysynaptic activation. This idea is supported by the fact that the responses with longer latencies also had more latency-variation, as revealed from the width of the peaks in the PSTHs.

The occurrence of responses of the same cell with spikes at each of the two latencies was also reported for a few units in rat and cat. Here again it is likely that the fo/fi stimulation leads to the activation of two pathways, one mono- and the other di- or polysynaptic. Finally, for the small number of units that responded to stimulation of the subiculum, we found long latencies with large variation (not shown), indicating that here also polysynaptic activation took place. In conclusion, we can say that there exists a population of output neurons in the Acb which can be activated by direct inputs from the subicular cortex, and by longer loops as well. The latter do not necessarily have to be located within the Acb itself. In the present study it was found that for the spontaneously active cells more than 40% of the units that responded to either fo/fi or subiculum stimulation were inhibited. Inhibitory responses have been reported for the rat and for the rabbit. This type of response was found for 17% to 25% of the responding units. From the anatomy of the intrinsic circuitry it is most likely that monosynaptically activated GABAergic (projection) cells have recurrent collaterals that make synaptic contacts within the Acb, thus inhibiting other cells in adjacent regions. Regarding the distribution of inhibitory responses following fo/fi stimulation it appears that these occur mainly in the shell region. Yang and Mogenson already showed that the occurrence of inhibitory responses was restricted to the medial and ventral aspects of the Acb, but these authors did not distinguish between the two partitions as we did in the present study.

The latencies of the antidromic action potentials following VP stimulation are in good agreement with the results of Yang and Mogenson. They recorded 55 units in the medial Acb and found latencies in the range of 4–10 ms (mean 7 ms). The seven excitatory units that gave orthodromic responses to VP stimulation were probably activated by projections from the pallidal area to the Acb. It is of interest to mention that orthodromic responses in the Acb following stimulation of the globus pallidus have been reported.

Inhibitory responses following VP stimulation may have been the result of activation of a collateral of a projection cell as mentioned in the previous paragraph. Presumably, this was the case for 56% of the small sample of nine units that responded to stimulation of the limbic input as well as the pallidal area. We cannot exclude, however, that the inhibition following VP stimulation could have been mediated by a direct GABAergic projection from the VP to the Acb.

Paired-pulse facilitation

The pronounced paired-pulse facilitation described here for the evoked potentials recorded in the Acb for intervals of 80 to at least 200 ms has to the best of our knowledge never been investigated. For the shorter intervals, the results can be compared with only one other study of the same kind. DeFrance et al. recorded paired-pulse responses at intervals less than 40 ms. They found a depression as was also the case...
in the present study for intervals between 20 and 40 ms.

The findings of the field potentials are supported by the results of the single unit recordings. From the previous section it became clear that the probability of firing following fo/fi stimulation was related to the P1 and P2 components. Paired-pulse facilitation was clearly present in all units with excitatory responses.

Similar phenomena have been described in the dorsal striatum following stimulation of the cortico-striatal pathways.20 These authors suggest that the mechanism underlying this form of short-term plasticity is a prolonged disinhibition. Indeed we found that the duration of the suppression of spontaneous activity of single units could last from 40 to 174 ms. If these inhibited cells in turn would send GABAergic fibres to another population of neurons, then these target cells may become disinhibited for about the same interval as just mentioned. A more simple explanation can be given by assuming that, following the conditioning stimulus, residual Ca2+ ions in the presynaptic terminal facilitate transmitter release following the test pulse, as was originally postulated for the neuromuscular junction.18 Another hypothesis that should be kept in mind is that this facilitation may be mediated through glutamate N-methyl-D-aspartate (NMDA) receptors. Recently, paired-pulse phenomena of the same type were found in an in vitro slice preparation of the Acb.21 The enhanced responses could, at least in part, be suppressed by application of D-(-)-2-amino-5-phosphonopentanoic acid, a NMDA receptor antagonist. This receptor type has been related to other forms of synaptic plasticity in the vertebrate central nervous system.3 It is also of interest to note that the intervals at which paired-pulse facilitation was encountered correspond to frequencies in the range from about 5 to 12 Hz. This frequency range is similar to that of the septo-hippocampal theta rhythm of the rat.32 Therefore, the suggestion may be made that neuronal responses of the Acb may be facilitated when theta rhythmic activity is present in the hippocampal formation.

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