J. Physiol. (1986), **379**, *pp.* 309–330
With 18 *text-figures*
Printed in Great Britain

PROPERTIES OF RAT MEDIAL SEPTAL NEURONES RECORDED *IN VITRO*

BY MENAHEM SEGAL
From the Center for Neurosciences, The Weizmann Institute of Science, 76100 Rehovot, Israel

(*Received 21 August 1985*)

SUMMARY

1. Activity of neurones of the rat medial septal nucleus (m.s.) was recorded in *in vitro* slice preparations. The recorded population could be divided into low (< 30 MΩ)- and high-input-resistance (> 30 MΩ) neurones. The high-resistance neurones tended to fire spontaneous action potentials and post-synaptic potentials.

2. Some of the spontaneously active cells fired rhythmically at rates of 2–10 Hz. The rhythmicity disappeared following hyperpolarization of the recorded cell. The cells could fire repetitive Ca^{2+} spikes in the presence of tetrodotoxin (TTX) and intracellular Cs^{+}. Cd^{2+} blocked this rhythmicity.

3. Most of the m.s. cells had a non-linear voltage–current relation in both the hyperpolarizing and depolarizing directions. Hyperpolarizing rectification was selectively blocked by extracellular Cs^{+} whereas depolarizing rectification could be blocked by TTX.

4. A recovery from hyperpolarization was associated in many cells with a transient depolarization (anodal break (a.b.) potential). A 20 ms 15 mV hyperpolarization could trigger an a.b. potential. The a.b. potential was reduced by TTX and Cs^{+} but not by Cd^{2+} or Mn^{2+}.

5. Depolarization of quiescent neurones triggered action potential discharges. A common pattern of discharge was a burst of two spikes which kept a fairly constant interspike interval. The second spike in a doublet could not follow a rate of 10 Hz depolarizing current pulses. It was also sensitive to topical application of Cd^{2+}. It is therefore suggested that Ca^{2+} might be involved in the generation of the doublet.

6. Long depolarizing current pulses produced trains of action potentials, showing little accommodation and little after-hyperpolarization, indicating that these cells possess little Ca^{2+}-dependent K^{+} current.

7. Many cells emitted spontaneous post-synaptic potentials at high rates. These could be blocked by picrotoxin. Stimulation of the lateral septal (l.s.) nucleus produced a Cl^{-}-dependent i.p.s.p. The i.p.s.p. was blocked by picrotoxin. Topical application of γ-aminobutyric acid (GABA) produced a marked Cl^{-}-dependent increase in conductance. It is suggested that l.s. projects a GABA-mediated inhibitory connexion to the m.s.

8. Acetylcholine (ACh) depolarized m.s. neurones and caused an increase in input resistance. The response was present in TTX or Cd^{2+}-containing medium. Atropine blocked responses to ACh.

9. 5-hydroxytryptamine (5-HT) hyperpolarized m.s. neurones in a manner consistent with an increase in K^+ conductance. The effects of 5-HT were seen in TTX- and Cd^{2+}-treated m.s. slices.

10. It is suggested that the m.s. generates rhythmic activity spontaneously and that two major inhibitory afferents, a GABA-mediated and a 5-HT-mediated pathway, modulate this activity.

INTRODUCTION

The medial septum (m.s.). constitutes a major modulatory afferent system to the hippocampus. Stimulation of the m.s. can trigger slow rhythmic hippocampal activity and its lesion can abolish this rhythm (Gray & McNaughton, 1983; Seifert, 1983; Buzsaki, Leung & Vanderwolf, 1984). M.s. neurones fire *in vivo* in close correlation with waves of hippocampal rhythmic activity (Gogolak, Stump, Petsche & Sterc, 1968; Macadar, Roig, Monti & Budelli, 1970; Apostol & Creutzfeldt, 1974). The physiological and pharmacological properties of m.s. neurones have been studied extensively in the intact brain (Petsche, Stump & Gogolak, 1962; Macadar *et al.* 1970; McLennan & Miller, 1974; Segal, 1976; Assaf & Miller, 1978; Lamour, Dutar & Jobert, 1984). The cells possess a characteristic bursting firing pattern that can be entrained by stimulation of the fimbria. They respond with a fairly short latency to sensory stimulation, especially to noxious ones (Segal, 1973; Lamour *et al.* 1984). They receive potent inhibitory connexions from brain-stem serotonin and noradrenaline-containing nuclei (Segal, 1976; Assaf & Miller, 1978). A recent intracellular study indicates that they also receive a local inhibitory input (Dutar, Lamour & Jobert, 1985).

The mechanisms underlying the unique properties of m.s. neurones remain unknown. One important question is whether the neurones generate the rhythmic activity intrinsically or whether they are triggered by afferent input. A partial answer to this question is given in the study of Vinogradova, Brazhnik, Karanov & Zhadina (1980) suggesting, on the basis of an extracellular study in an *in vitro* slice preparation, that the rhythmic activity is intrinsic to the septum. Many, but not all (see Baisden, Woodruff & Hoover, 1984; Wainer, Levey, Rye, Mesulam & Mufson, 1985), of the septohippocampal neurones contain acetylcholine (ACh) and constitute a source of the rich cholinergic innervation of the hippocampus. Indeed, cholinergic drugs interfere with the generation of rhythmic hippocampal activity (Buzsaki *et al.* 1984). It is of interest to find out whether the mechanism of action of ACh in m.s. neurones is different from its action on cholinoceptive neurones. The present study examines some physiological and pharmacological properties of m.s. neurones recorded in *in vitro* slice preparations.

METHODS

Young (1·5 month old) Wistar male rats were decapitated, their brains rapidly removed and placed in cold (4 °C) Krebs solution. A block of tissue containing the septal area was trimmed with a razor blade, placed on a stage of a Vibroslice (F. Haer) and sliced into frontal 400 μm slices.

The block was cut at a slight (15 deg) angle so that the ventral edge of the slice was more rostral than the dorsal edge. The slices were placed in an interface chamber where they were perfused with warm (35 °C) Krebs solution and exposed to a constant flow (0·4 l/min) of warm, humidified 95% O_2/5% CO_2 gas mixture. The Krebs solution contained 124 mM-NaCl, 2 mM-$CaCl_2$, 2 mM-$MgSO_4$, 5 mM-KCl, 10 mM-glucose, 1·25 mM-KH_2PO_4 and 25 mM-$NaHCO_3$. The medium was saturated with the O_2/CO_2 gas mixture and had a pH of 7·4. Drugs were prepared in the recording medium immediately before use and applied by the microdrop technique (Segal, 1980, 1982). 0·2–0·5 mM-Cd^{2+} and 5 mM-Ba^{2+} were prepared in a medium buffered with 5 mM-HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid) to avoid precipitation. HEPES-buffered medium was used in these cases as a control solution. Recording was made with 1·2 mm o.d. glass-fibre-filled pipettes pulled with a Brown Flaming pipette puller. The micropipettes were filled with 3 M-NaCl, 3 M-KCl, 3 M-CsCl, 4 M-potassium acetate or 4% Lucifer Yellow (LY, Li salt, Sigma). Signals were amplified with a Dagan 8100 single-electrode amplifier. Hyperpolarizing current pulses were used routinely to estimate input resistance of the cell. Care was taken to assure a balanced bridge throughout the experiment, especially when, in addition to the current pulses, there was an application of d.c. current to change membrane potential. The current pulses were of 50 or 100 ms duration to allow voltage deflexions to reach a steady state. Signals were continuously plotted on a 2400 Brush recorder.

RESULTS

Stable intracellular recording was made from a population of eighty-eight m.s. neurones. Stability of recording over a period of at least 15–20 min, as well as an action potential larger than 80 mV and input resistance of at least 10 MΩ, were the main criteria for inclusion in the recording sample. The input resistance of the recorded neurones was estimated from the linear portion of a voltage–current relation in the hyperpolarizing direction. Care has to be taken to avoid the rectifying regions of the voltage–current curve (see below). The input resistance of the cells was distributed bimodally. There were cells with low input resistance (10–30 MΩ, mode = 25 MΩ) and cells with high input resistance (30–150 MΩ, mode = 70 MΩ). Some properties described below were associated with the input resistance of the cells and some were seen in most cells of both types. A sample of fifteen cells stained with LY, not included in the reported population of neurones, indicated that the low-resistance neurones might be the larger rostral m.s. neurones while the higher-resistance neurones are probably the smaller more caudal neurones. Many cells of both types are likely to be cholinergic since the recording region is rich in neurones containing acetylcholinesterase (AChE) (Fig. 1). No attempt was made in the present study to double-label cholinergic neurones with LY.

The resting membrane potential, estimated upon withdrawal of the recording electrode from the neurone, was similar in both groups of neurones and amounted to $-64\pm1{\cdot}1$ mV (mean ± S.E. of mean, $n = 38$). The action potential size was $106\pm2{\cdot}1$ mV ($n = 14$) in the low-resistance neurones compared to $98 = \pm1{\cdot}8$ mV ($n = 24$) in the high-resistance neurones. In both groups the action potentials were rather fast (1–2 ms). The membrane time constant, estimated from the semilogarithmic plots of the time course of a voltage response to a small hyperpolarizing current pulse, was $17\pm1{\cdot}5$ ms ($n = 10$). There was no clear difference in this parameter between the two cell groups. Many of the m.s. cells discharged action potentials spontaneously. This was seen more frequently in samples of the high-resistance (twenty-one of twenty-two cells) than the low-resistance neurones (five of twenty-one cells) recorded with KCl-containing micropipettes. Of the spontaneously firing neurones some

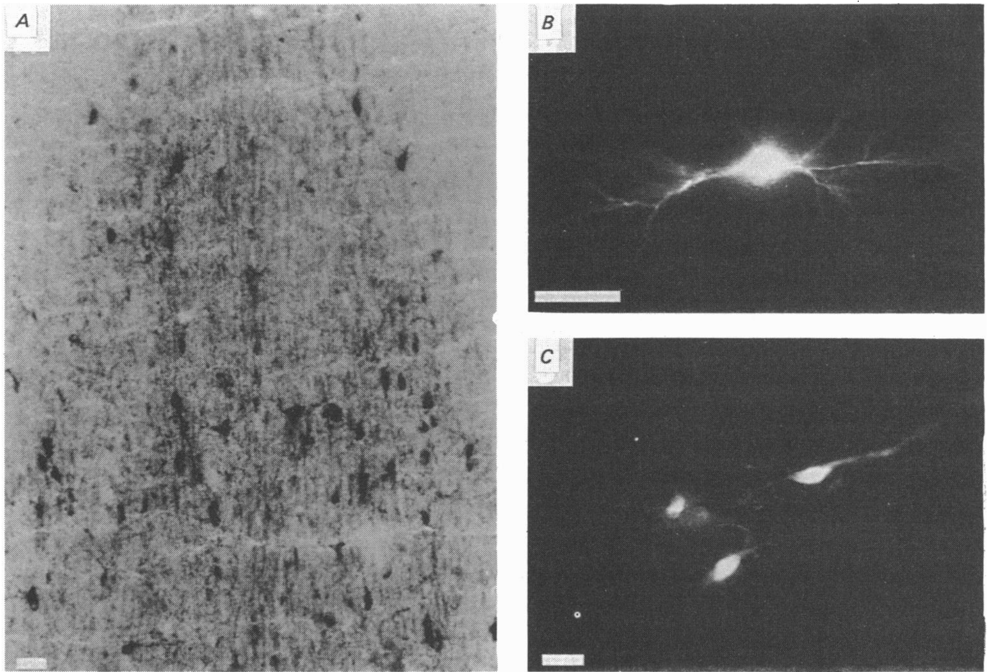

Fig. 1. *A*, 50 μm frontal section through the medial septum (m.s.) stained for AChE (Butcher, 1978) to illustrate the abundance of cholinergic neurones near the mid line, where most of the recordings were made. *B*, Lucifer-Yellow-filled neurone. A low-resistance neurone found in the rostral part of the m.s. *C*, dye-coupled, small neurones found in the main body of the m.s. Only one of these neurones was filled with the Lucifer Yellow dye. Calibration 50 μm.

discharged rhythmically at rates of 2–10 Hz (Fig. 2*A* and *B*). These discharges were not a result of cell injury as they were maintained over long periods of observations without degeneration of the membrane potential or input resistance of the cell and could also be seen with extracellular recording micropipettes (Fig. 2). Some of the neurones fired rhythmically in bursts of two spikes reminiscent of their *in situ* counterparts (Fig. 2*C*). Hyperpolarizing the cells so that they ceased to fire action potentials did not reveal an underlying rhythmic synaptic activity. It should be noted that a large number of the spontaneously active cells also exhibited a high rate of spontaneous post-synaptic potentials yet these too could not account for the rhythmic spontaneous activity of the recorded neurones (see below).

Rectification

Several physiological properties were detected in most neurones in response to passage of current pulses. A large hyperpolarizing current pulse resulted in a pronounced inward rectification (Purpura, Prelevic & Santini, 1968; Halliwell & Adams, 1982). This was developed over a period of 30–50 ms at a potential about 15 mV negative to rest and remained constant throughout the hyperpolarized interval. In fact, the large rectification can be illustrated best when the input

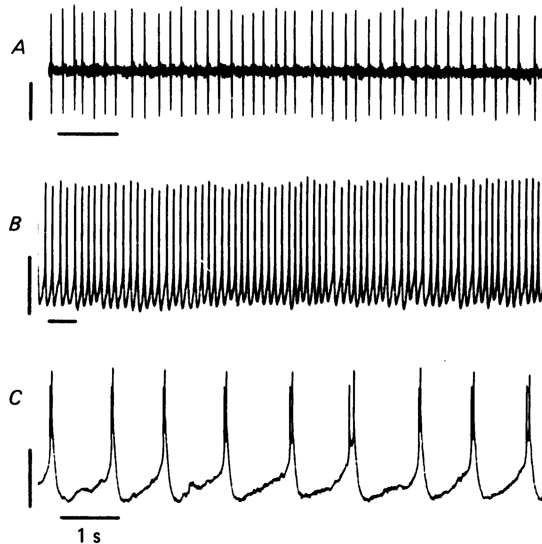

Fig. 2. M.s. neurones are spontaneously active. *A*, extracellular recording with a NaCl micro-electrode illustrates rhythmic activity at a rate of 6 Hz of a m.s. neurone. *B*, intracellular recording from a spontaneously active neurone, firing at a rate of 4 Hz. *C*, spontaneously bursting neurones firing bursts of two action potentials at regular intervals. Time base is 1 s for all records. In *B* and *C* the spike is truncated by the frequency response of the recorder. Calibration 1 mV (*A*), 20 mV (*B* and *C*).

resistance was measured in response to small hyperpolarizing current pulses and the cell was current-clamped to different potentials (Fig. 3). Input conductance in the presence of the inward rectification in the cell illustrated in Fig. 3 changed from 11 to 27 nS across about 30 mV of membrane potential. The rectification could be blocked by a microdrop application of 5 mM-Cs^{+} (Fig. 4). Other drugs tested (1 μM-tetrodotoxin (TTX) and 1 mM-Cd^{2+}) did not mimic the effects of Cs^{+} (see below). It thus appears that the inward rectification involves primarily (but perhaps not exclusively (see Halliwell & Adams, 1982)) an influx of K^{+} into the recorded neurones.

A small inward rectification could be seen in many neurones in response to a depolarizing current pulse. This was difficult to study under normal conditions because most neurones either fired spontaneously or in response to very small depolarizing current pulses thus preventing the analysis of the time course and voltage dependence of the depolarizing rectification. The cells could however be current-clamped at depolarized potentials and the responses to small hyperpolarizing current pulses examined (Figs. 3 and 5*A*). This procedure uncovered the presence of a significant rectification exhibited by an apparent 30–60% increase in input resistance when the cell was depolarized by about 5–10 mV from rest. A qualitatively similar rectification has been observed in other neurones and ascribed to a steady Na^{+} current (Stafstrom, Schwindt, Chubb & Crill, 1985; French & Gage, 1985). We have examined this possibility by topical application of Cd^{2+} or Mn^{2+} to block Ca^{2+} currents, or TTX to block voltage-dependent Na^{+} currents. In most cases examined

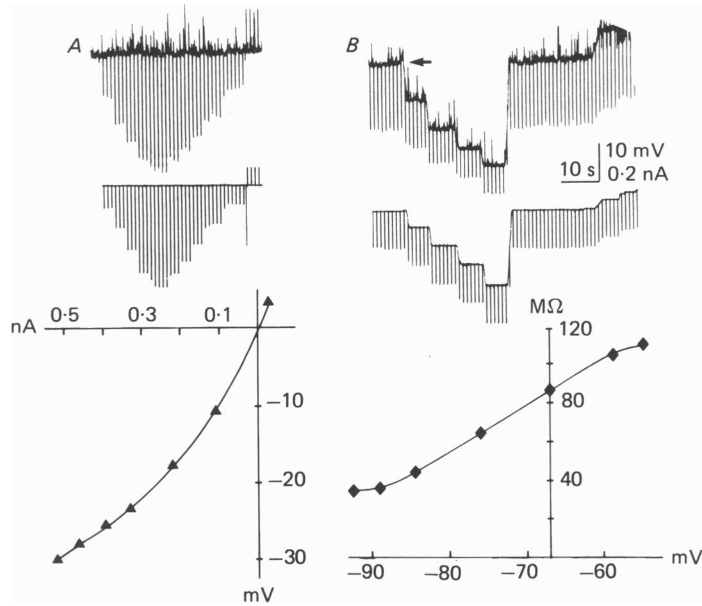

Fig. 3. Rectifying properties of m.s. neurones. *A*, the cell was held at resting potential (−67 mV) and 50 ms hyperpolarizing or depolarizing current pulses were applied through the electrode. The current–voltage relation is linear only when the cell is hyperpolarized by 10–15 mV from rest. Input conductance increases at more polarized potentials. Depolarizing current pulses generated action potentials and were not useful for estimating input resistance of the cell. *B*, same cell, current-clamped at different potentials. Hyperpolarizing current pulses were applied repetitively to estimate the input resistance of the cell. Input resistance of the cell was reduced markedly when the cell was hyperpolarized and increased by depolarization.

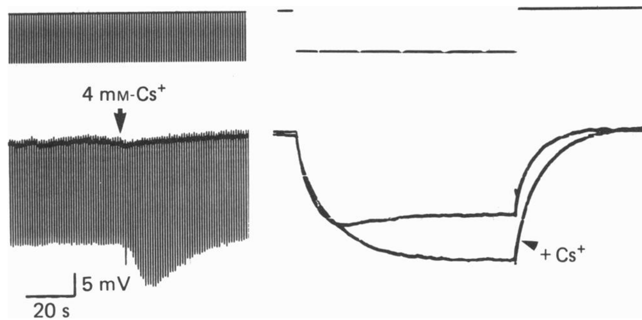

Fig. 4. Cs^+ blocks inward rectification. Hyperpolarizing 0·5 nA current pulses, 100 ms in duration, were applied at a rate of 1 Hz to a m.s. cell. Topical application of 4 mM-Cs^+ caused a transient increase in input resistance. A close examination of the voltage response to the current pulse (right) indicates that in the presence of Cs^+ the sag in the voltage is replaced by an exponential response to the current pulse, thus adding to the apparent input resistance of the cell. Resting membrane potential of the cell = −60 mV.

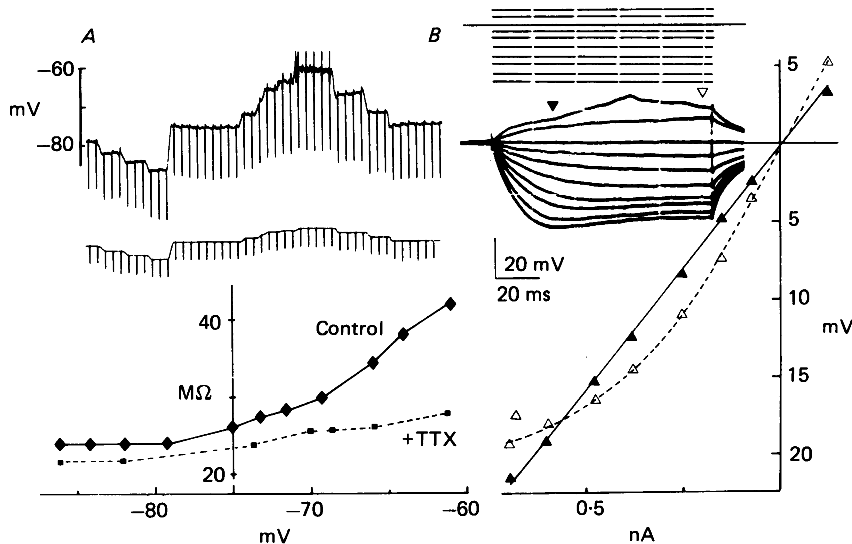

Fig. 5. *A*, depolarization-induced rectification is blocked by TTX. A low-resistance (25 MΩ) high-resting-membrane potential (−75 mV) neurone having little hyperpolarizing rectification was depolarized by passage of d.c. current to reveal an apparent increase in input resistance associated with anodal break discharges (spikes truncated). In the presence of 1 μM-TTX the input resistance of the cell was reduced, especially in the depolarized region (bottom). *B*, current–voltage relations of TTX-treated m.s. neurone. Hyperpolarizing and depolarizing current pulses of varying magnitudes were applied for a duration of 100 ms. The initial response, measured 30 ms after the onset of the current pulse demonstrated a linear current–voltage curve (▼). The later part (▽) exhibits a pronounced rectification. Note that a depolarizing pulse produced a small, regenerative potential. Resting membrane potential = −65 mV.

(twelve cells), TTX could block the steady-state depolarizing rectification (Fig. 5*A*), while it had no effect on the time-dependent hyperpolarizing rectification (Fig. 5*B*). In the presence of TTX (Fig. 5*B*) a small regenerative potential could be detected which was distinguishable from the steady-state depolarizing rectification. Ca^{2+} antagonists, 5 mM-Mn^{2+} or 0·5 mM-Cd^{2+}, did not affect the depolarizing rectification.

Anodal break potential

In neurones of both types (33 % of the low-resistance and 54 % of the high-resistance cells examined) there was a distinct transient (20–50 ms) 1–5 mV depolarizing response upon recovery from hyperpolarization. When this transient response exceeded 5–7 mV it caused the discharge of an action potential. This overshoot was similar to anodal break (a.b.) potentials seen elsewhere (e.g. Jahnsen & Llinas, 1984) and will be referred to herein as a.b. potential. The activation properties of the a.b. potential were studied in seven cells, by application of priming hyperpolarizing pulses of various magnitudes and durations (Fig. 6). A 10 mV hyperpolarization was sufficient to evoke a detectable a.b. potential. The size of the a.b. potential was correlated with the duration of the priming hyperpolarizing pulse and was detected with a pulse as short as 20 ms. To evoke an a.b. response the potential had to be

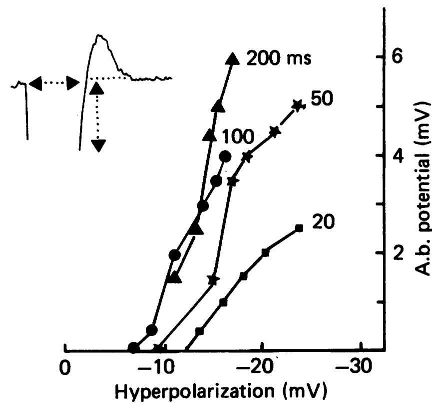

Fig. 6. Activation properties of anodal break (a.b.) potential. Inset illustrates the independent variables: the duration and magnitude of the hyperpolarizing current pulses. The size of the a.b. potential is dependent on both the duration and magnitude of the priming hyperpolarization. A short pulse of 20 ms that hyperpolarizes the membrane by about 15 mV is sufficient to evoke a threshold a.b. potential. Inward rectification is not seen under these conditions.

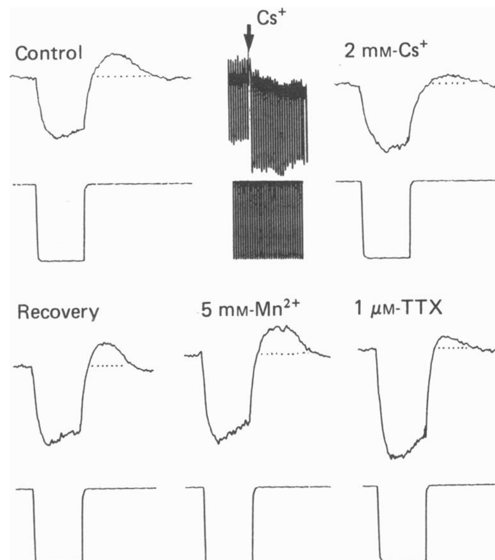

Fig. 7. Drug effects on a.b. potentials. Successive tests were done on the same cell. A 100 ms 0·3 nA hyperpolarizing current pulse was applied to the cell at a resting membrane potential of −58 mV. The recovery from the hyperpolarization was associated with a 5 mV a.b. potential. Cs^+ (2 mM in a microdrop) temporarily antagonized the a.b. potential. Mn^{2+} (5 mM in a microdrop) did not affect the a.b. potential while TTX reduced it. TTX did not affect the inward rectification recorded in this cell. The change in input resistance across the experiment (1 h) is probably due to a spontaneous improvement in the recordability of the cell.

brought back to about resting level. No a.b. was seen when the membrane was hyperpolarized by about 10 mV from rest but a larger a.b. was seen in a depolarized cell. The possible ionic mechanisms underlying the a.b. potential were analysed using drugs that selectively interfere with some ionic conductances. Previous studies have suggested that a similar potential is mediated by a subthreshold Ca^{2+} current (Jahnsen & Llinas, 1984). Cd^{2+} (1 mM) or Mn^{2+} (5 mM) in a dose that otherwise blocks synaptic potentials were unable to modify the a.b. potential (Fig. 7). We observed that in some cells the a.b. potential was associated with the presence of the hyperpolarizing rectification, i.e. the cells that recover from the rectification would overshoot. This does not imply that the a.b. potential simply reflects a tail current of the inward rectifier, since they appear to have different activation potential ranges and time courses. For example, no rectifications were seen with a 10 mV or a 20 ms hyperpolarizing current pulse. The possible involvement of K^+ and Na^+ in the a.b. potential was examined with topical applications of Cs^+ (1–5 mM) and TTX (Fig. 7). Cs^+ caused a marked reduction in a.b. potential along with the other effects seen above (Fig. 4). TTX, which blocks the depolarizing rectification, also caused a marked reduction in the a.b. potential (Fig. 7). These experiments indicate that the a.b. potential might involve a Na^+-dependent steady current that is activated at rest and depolarized potentials. The Na^+ current might have a faster activation rate than the K^+ current, resulting in a transient depolarization. Voltage-clamp experiments are needed to substantiate this possibility.

Depolarizing responses

In response to a small depolarizing current pulse many m.s. neurones discharge a burst of two action potentials (Fig. 8*A*). Within certain depolarizing current limits the spike doublet maintained a constant interspike interval (5–8 ms). The current threshold to evoke a doublet was nearly identical with that needed to evoke a single spike, and was much lower than that needed to evoke three spikes for a given duration (usually 50 ms). The second spike in a doublet had usually a smaller amplitude (by 10–20%) than the first one and was slightly broader as well. However, there are distinct differences between the doublets recorded here and the bursts evoked in hippocampal neurones (Wong & Prince, 1978). Hippocampal bursts consist of four to six spikes of decreasing amplitudes which ride on a large depolarizing wave resulting probably from activation of Ca^{2+} current, and are followed by a burst after-hyperpolarization. The doublets in m.s. neurones do not ride on a large depolarizing potential and are not followed by a large after-hyperpolarization. Nevertheless, the dependence of the doublet on Ca^{2+} is indicated in several types of experiments. First, the occurrence of a doublet is markedly dependent on the repetition rate of the depolarizing pulse; at repetition rates slower than 1 Hz the doublet occurred in all of the responses to a depolarizing pulse whereas at 10 Hz the second spike in the doublet was nearly absent (Fig. 8*B*). This is unlikely to have been caused by an increase of intracellular Na^+ concentration since these cells can fire at high rates with little accommodation (see below). More likely, the doublet might be blocked by accumulation of intracellular Ca^{2+}. Secondly, the doublet was blocked in four of seven cells tested by topical application of Cd^{2+} (1 mM) (Fig. 9) which did not affect the threshold and duration of the first spike. Thirdly, in the presence of Ba^{2+}

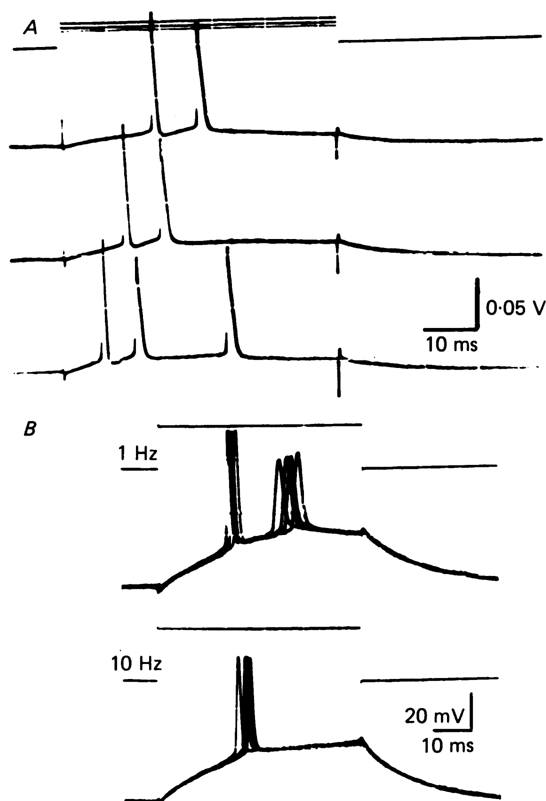

Fig. 8. *A*, a typical response of a m.s. neurone to a series of depolarizing current pulses. At threshold, top trace, two action potentials ('a doublet') are discharged with an interspike interval of 9 ms. A larger current pulse (middle trace) reduced the latency of the doublet from 18 to 12 ms but did not recruit more action potentials. A still larger current pulse, bottom trace, reduced further the latency of the doublet to 7·5 ms and recruited a third, long-latency spike. Resting membrane potential = −62 mV. *B*, the doublet is sensitive to frequency of application of depolarizing pulses. A constant depolarizing 50 ms current pulse is applied at a rate of 1 or 10 Hz. Note that the second spike in a doublet has a lower amplitude than that of the first one and has a variable latency. The second spike is absent when the pulse is applied at a rate of 10 Hz. No other clear effects of the high-frequency stimulation are evident.

(5 mM in a droplet) the second spike was markedly prolonged (Fig. 9). Ba^{2+} passes better than Ca^{2+} through Ca^{2+} channels and blocks Ca^{2+}-dependent K^+ currents (Hagiwara, 1973).

The second spike in a doublet could occasionally rise off a large depolarizing after-potential (d.a.p.) (Kandel & Spencer, 1961) which can be detected in some quiescent neurones following a brief depolarization which triggers an action potential. The d.a.p. is not a common property of most spontaneously active m.s. neurones. The d.a.p. was generated as an all-or-none response to produce a transient 10–16 mV depolarization (Fig. 10). However, it was not directly responsible for the generation

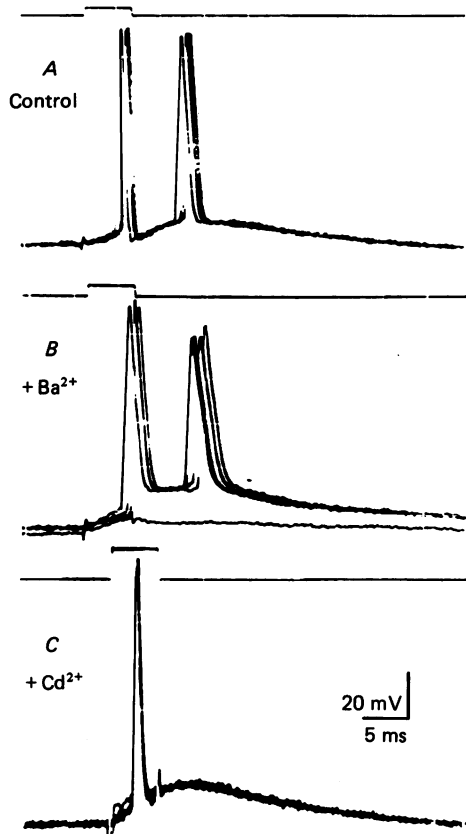

Fig. 9. Ion effects on action potential discharges of m.s. neurone. *A*, in the control condition a short, 5 ms depolarizing current pulse triggers the firing of a doublet. *B*, in the presence of 5 mM-Ba^{2+}, applied via a microdrop, the spikes are broader and they lose their fast hyperpolarizing after-potentials. Cd^{2+} (*C*) (1·0 mM in a microdrop) eliminates the second spike in the doublet and reveals an underlying depolarizing after-potential.

of a doublet as it was not modified by high-frequency stimulation (Fig. 10) which totally blocked the doublet. The mechanisms underlying the d.a.p. have not been analysed in the present study. The persistence of the d.a.p. in high-frequency stimulation rate and its resistance to Cd^{2+} (data not shown) indicate that it is not dependent on Ca^{2+}.

Application of a long (0·5 s) depolarizing current pulse evoked a train of action potentials in twenty-two recorded neurones. The frequency of action potentials was proportional to the amount of current passed (Fig. 11). In all cells recorded a clear distinction could be made between the initial and the steady-state rates of action potential discharges; the initial rate was always faster than the steady-state one (Fig. 11). Only a small accommodation could be recorded, i.e. the cells did not cease to fire action potentials even during long depolarizing current pulses. In some cells the depolarizing current pulse evoked repetitive burst activity similar to that recorded in some spontaneously active cells (see Fig. 2). In the hippocampus, as in the other neurone types, the termination of a depolarizing current pulse is followed by a large

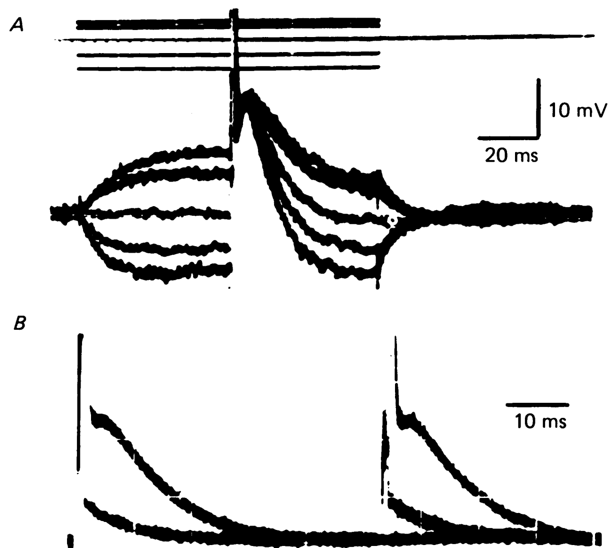

Fig. 10. Depolarizing after-potential (d.a.p.) in a m.s. neurone. *A*, an action potential was evoked by passage of a 1 ms depolarizing current pulse. The membrane potential was varied by passage of 100 ms depolarizing and hyperpolarizing current pulses. Although a clear reversal was not recorded in this experiment, the null potential appears to be 16 mV depolarized to rest regardless of the size of the conditioning potential. *B*, the d.a.p. can follow high stimulation rates without a loss in its magnitude. The cell, same as in *A*, was stimulated repetitively at a rate of 20 Hz. The true d.a.p. can be measured by subtracting the response in the presence and absence of a spike. This has yielded no decrement in the d.a.p. over the repetitive stimulation.

and long-lasting after-hyperpolarization associated with a marked increase in conductance. This after-hyperpolarization is caused by activation of a Ca^{2+}-dependent K^+ current and is blocked by Ca^{2+} antagonists (Cole & Nicoll, 1984). In a random sample of hippocampal neurones recorded under the same conditions and having a similar resting potential, the after-hyperpolarization amounted to $-5{\cdot}1 \pm 0{\cdot}4$ mV (mean $\pm$ s.e. of mean, $n = 12$ observations). In the present m.s. neurones, application of a large depolarizing pulse resulted in a train of action potentials which was followed by hyperpolarization of only $-2{\cdot}9 \pm 0{\cdot}31$ mV ($n = 22$). It appears therefore that m.s. neurones possess a smaller capacity to activate a Ca^{2+}-dependent K^+ current than their hippocampal counterparts.

Rhythmicity

As indicated, many m.s. neurones fire rhythmically yet their hyperpolarization by current does not reveal any rhythmic synaptic activity. This observation might be misleading since hyperpolarization also activates anomalous rectification, which increases the input conductance of the cell and might thus rectify any rhythmic synaptic current. The possible synaptic origin of the rhythmic oscillations of the membrane were therefore studied in cells isolated from afferent neurones with TTX. Under these conditions circuit activity would be blocked and any rhythmic activity

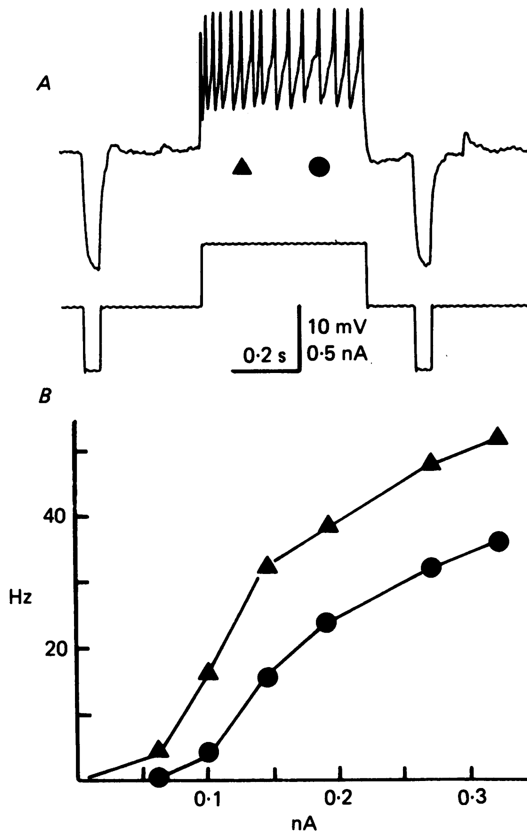

Fig. 11. M.s. neurones show little accommodation and train after-hyperpolarization. *A*, the cell was depolarized by a 0·5 s current pulse and the number of spikes generated in the first and second halves of the pulse counted and plotted as a function of the magnitude of the current pulse (*B*). In addition, hyperpolarizing current pulses were applied before and after the depolarization to assess the changes in input resistance of the cell. The changes in resting membrane potential following the train were measured as well. Even a large current pulse that caused a discharge of 30 spikes/s (*A*) produced only 1·5 mV of after-hyperpolarization.

should be intrinsic to the recorded neurone. Experiments were conducted with CsCl micropipettes which leak Cs^+ into the cell to block outward K^+ currents. These conditions promote detection of spontaneous or evoked Ca^{2+} currents. Six Cs^+-loaded neurones recorded in TTX-treated tissue could fire Ca^{2+} spikes repetitively in a highly rhythmic pattern for long durations. As seen in Fig. 12 the Ca^{2+} spike had a rapid rising phase followed by a slow decay and a long plateau potential. Following the plateau potential the cell returned to resting potential and began a gradual depolarization phase which led to a new action potential. This rhythmic manner was maintained for long recording intervals and was blocked by a Ca^{2+} antagonist, Cd^{2+} (Fig. 12).

The involvement of Ca^{2+} in the rhythmic activity seen in normal neurones (without TTX) was examined using the Ca^{2+} antagonist Cd^{2+} (five cells). In four of the cells

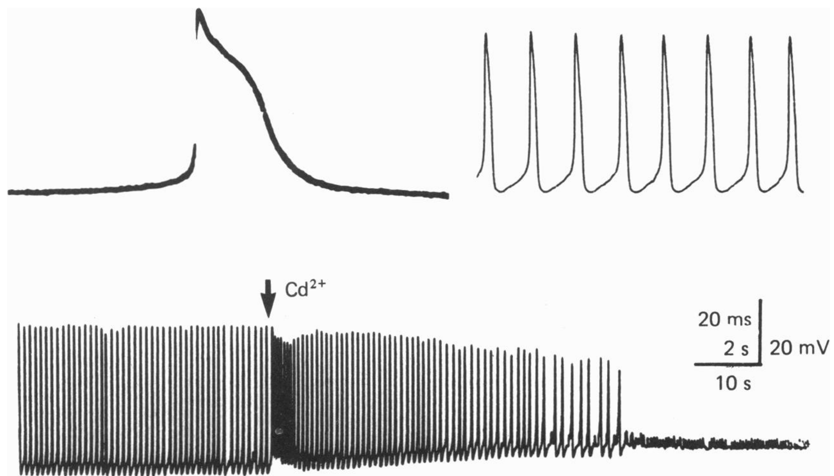

Fig. 12. Rhythmic activity can be recorded in m.s. neurones in slices treated with TTX. The cell was impaled with CsCl micro-electrodes which diffuses Cs^+ into the cell and blocks outward K^+ currents. A spontaneous action potential is depicted at the top left panel. The scale for the top left panel is 20 ms, top right 2 s and bottom panel 10 s. The spikes were discharged repetitively at a rate of 2 Hz which was maintained for long (at least 30 min) periods. Topical application of Cd^{2+} blocked the repetitive firing (bottom panel). Resting membrane potential = −65 mV.

tested, Cd^{2+} slowed or eliminated spontaneous activity, indicating that intrinsic Ca^{2+} currents may regulate rhythmic activity of m.s. neurones *in vitro*.

Synaptic mechanisms

Spontaneous post-synaptic potentials (s.p.s.p.s) were recorded in a large number of neurones. This activity could be seen best in the high-resistance neurones. To identify the possible origin of the s.p.s.p.s, the slice was stimulated electrically at various locations and the responses of the neurone to the stimulation examined. It was reasoned that the origin of s.p.s.p.s must be within the slice since they could be recorded even 10–12 h after the slice was cut, making it unlikely that detached terminals are still spontaneously active. The only location in the slice which could trigger consistently a p.s.p. in m.s. neurones was the lateral septal (l.s.) nucleus adjacent to the m.s. The p.s.p. had a 2–3 ms delay after l.s. stimulation, a fast rise time and a slow (30–40 ms) decay. A tetanic stimulation (50 Hz for 0·5 s) produced post-tetanic potentiation lasting for several seconds (data not shown) but no long-term effect could be recorded under these recording conditions. Using KCl micropipettes which load the recorded cell with Cl^-, the estimated reversal potential of the p.s.p. was about −20 mV. The reversal potential was not reached as the membrane potential was not shifted much in the depolarizing direction. With potassium-acetate-containing micropipettes, the p.s.p.s had a reversal potential of about −60 mV (Fig. 13). The correlation of the reversal potential with intracellular Cl^-concentration indicates that the p.s.p.s might be of an inhibitory type (i.p.s.p.s) and thus might be mediated by activation of a γ-aminobutyric acid (GABA) receptor.

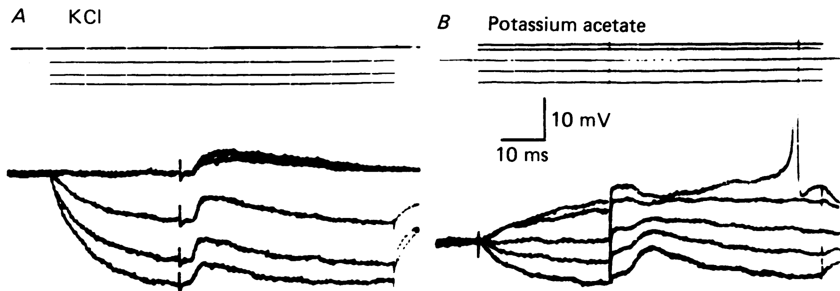

Fig. 13. Post-synaptic potentials recorded in m.s. neurone in response to electrical stimulation of the l.s. area. *A*, recording was made using KCl micropipette which loaded the cell with Cl^-. The p.s.p. was recorded at different membrane potentials. The reversal potential of the p.s.p. was extrapolated to −25 mV. *B*, p.s.p. recorded with a micropipette containing potassium acetate. The p.s.p. reverses at about 10 mV depolarized to resting potential. In both cases the stimulation parameters are: pulse duration 0·1 ms, amplitude 5–10 V monophasic stimulation with a stainless-steel micro-electrode.

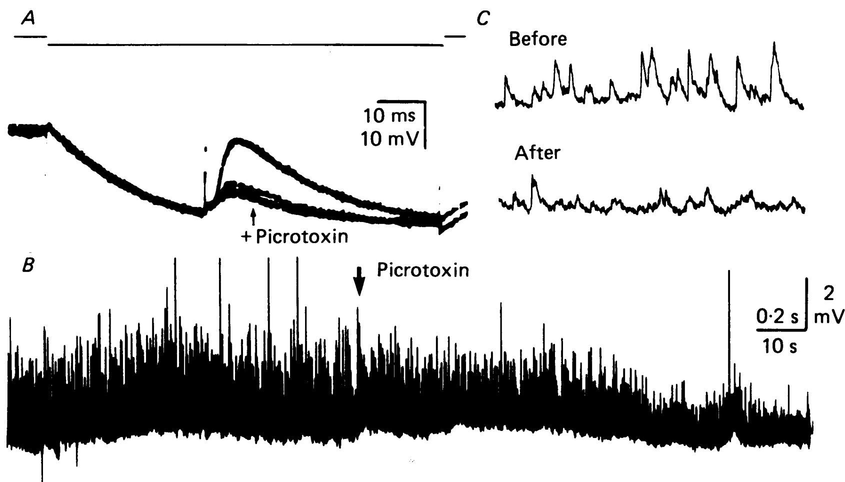

Fig. 14. Picrotoxin antagonizes spontaneous and evoked p.s.p.s. *A*, the l.s. is stimulated and the responses of a m.s. neurone are recorded intracellularly using a KCl-containing micropipette. The response is amplified by hyperpolarizing the membrane with a current pulse (top trace). In the presence of picrotoxin (arrowhead), the evoked p.s.p. is antagonized. *B*, spontaneous p.s.p.s recorded in another neurone with a KCl electrode. All p.s.p.s are depolarizing. After topical application of picrotoxin the magnitude of the s.p.s.p.s is reduced and the cell is slightly hyperpolarized. *C*, expanded time base (calibration 0·2 s in *B*) of the record in *B* to illustrate the size and frequency of the s.p.s.p.s before and after application of picrotoxin.

To test this possibility the GABA antagonist picrotoxin was applied near the recorded cell. Picrotoxin (5 mM-solution in a microdrop) blocked the evoked i.p.s.p.s as well as the s.p.s.p.s (Fig. 14). It is therefore likely that at least some of the recorded s.p.s.p.s are mediated by the action of GABA released from terminals of l.s. neurones.

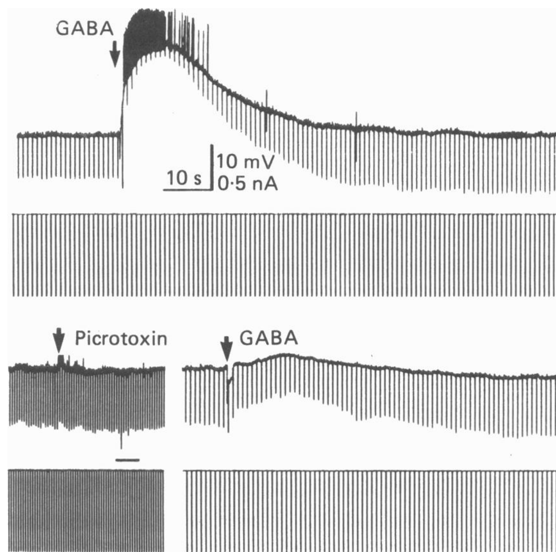

Fig. 15. Cellular responses to GABA. GABA (1·0 mM microdrop) was applied onto a m.s. neurone (arrowhead). The cell was depolarized by 18 mV and discharged action potentials. Hyperpolarizing current pulses (bottom traces), applied at a rate of 1 Hz to estimate input resistance of the cell, indicate that during recovery from the depolarization there is a 30 % increase in input resistance of the cell. Picrotoxin (bottom left) hyperpolarized the cell slightly and antagonized a later response to GABA.

Responses to putative neurotransmitter substances

The blockade of i.p.s.p.s and s.p.s.p.s by picrotoxin indicates that m.s. neurones may receive a potent GABA-mediated inhibitory input. The effects of topical application of GABA were studied in five m.s. neurones. GABA applied to Cl^--loaded neurones produced a 15–20 mV depolarization and a large increase in input conductance of these neurones. The marked increase in conductance associated with the initial response to GABA was replaced, during the recovery from the depolarization, by a long-lasting decrease in input conductance associated with no change in membrane potential of the recorded neurone (Fig. 15). The initial as well as the late responses were maximal with a microdrop containing the drug at 1 mM and were at threshold at 0·01 mM. At no concentration was there any apparent dual, fast action of the drug on the recorded neurones to indicate a soma–dendritic differentiation. Furthermore, topical application of picrotoxin blocked the responses to GABA and did not uncover a second hyperpolarizing component seen elsewhere (Andersen, Dingledine, Gjerstad, Langmoen & Laurson, 1980).

ACh

Significant AChE-containing fibres are present in the m.s. proper, indicating that these neurones might receive cholinergic innervation. This may arise from other cholinergic nuclei in the brain stem but also from axon collaterals of m.s. or adjacent diagonal band neurones (Swanson & Cowan, 1979). ACh produces an excitatory action

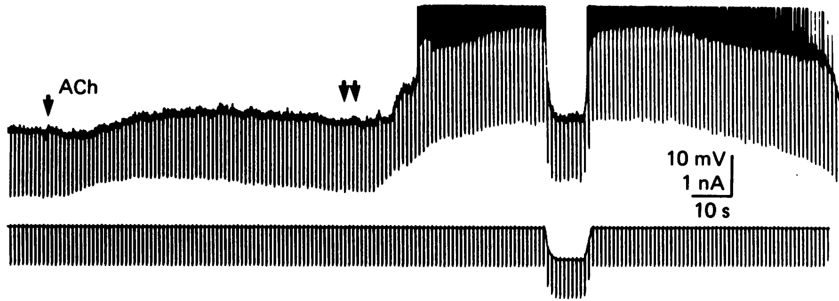

Fig. 16. Cellular responses to topical application of ACh. Hyperpolarizing current pulses were applied at a rate of 1 Hz to estimate the input resistance of the cell. Initially, a small microdrop of ACh was applied near the recorded neurone and produced an initial decrease in resistance followed by an increase and a small depolarization. A bigger microdrop (two arrows) produced a larger depolarization and an increase in action potential discharge rate. The membrane potential was brought back near the resting membrane potential by passage of current to reveal that the increase in resistance is a voltage-dependent process. The experiment was conducted in the presence of Cd^{2+} (1 mM) to suppress heterosynaptic effects. Recording was made with a KCl micro-electrode. Resting membrane potential = −65 mV.

on identified septo-hippocampal neurones (Lamour *et al.* 1984), yet it is not clear if this effect is mediated by the same ionic and pharmacological mechanisms as seen in post-synaptic neurones of the hippocampus (Cole & Nicoll, 1984; Segal, 1982). ACh was applied to twelve m.s. neurones and its effects on membrane potential and input resistance examined. Of the cells tested, four were pre-treated with TTX to eliminate possible detection of indirect effects of ACh. In all cells studied ACh produced a potent depolarization accompanied by an increase in action potential discharge rate (in TTX-free slices) and an apparent large increase in the input resistance (Fig. 16). This response was preceded by a short period (2–6 s) of a 20% decrease in resistance, as seen in hippocampal neurones (Segal, 1982). The decrease in resistance was also seen when the slice was bathed in TTX or Cd^{2+}, indicating that it is probably not mediated by a presynaptic action of ACh. The marked resistance increase which followed was only seen when the cell was already highly depolarized. To test whether the resistance increase does not reflect rectifying properties of the membrane, the cell was current-clamped back to the resting membrane potential. In about half of the tested cells the action of ACh reflected a voltage-independent increase in input resistance of the cell and was clearly seen at resting potential (e.g. Fig. 17). In the other cells (e.g. Fig. 16) the increase in resistance disappeared when the cell was repolarized to the region of the resting potential. The difference between the two types of responses was not correlated with the magnitude of the original depolarization or with resting properties of the cells. The voltage–current relation was measured by application of current pulses of varying magnitudes and plotted before and during the action of ACh. The voltage–current curves coincided at membrane potentials negative to −85 mV (Fig. 17). A true reversal potential could not be established. The responses to ACh were short-lasting and a recovery could be seen in most cases within a few minutes

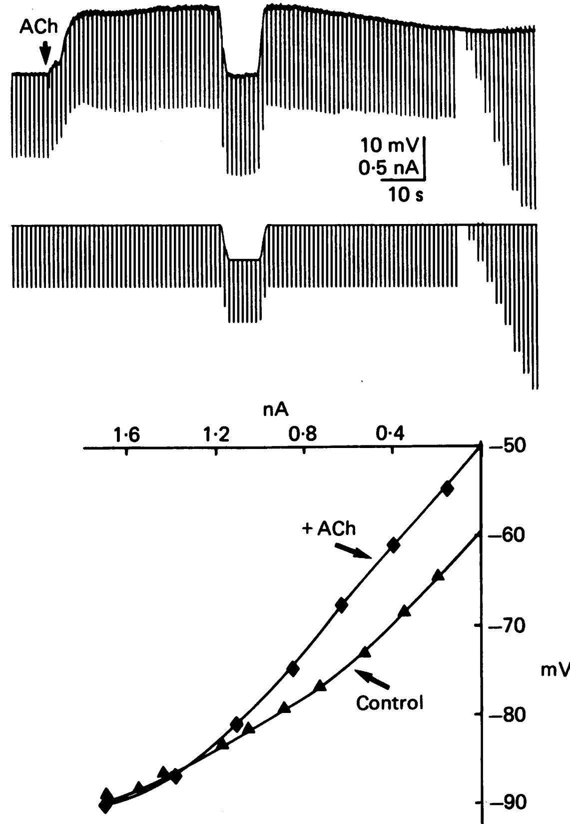

Fig. 17. Cholinergic responses of m.s. neurones are TTX insensitive. The slice was perfused with 10^{-6} M-TTX to block voltage-dependent Na^+ currents. ACh applied in a microdrop (arrowhead) produced a depolarization associated with a large increase in resistance. This resistance change was still pronounced when the membrane potential was pushed back to the resting level with current. A series of hyperpolarizing current pulses was applied during the action of ACh (top right) and before (not seen) to estimate the reversal potential of ACh action. This was plotted (bottom diagram) and revealed a null potential near K^+ equilibrium potential (−85 mV). A true reversal was not seen.

after ACh application, unlike the case in hippocampal neurones where the effects of ACh last for a longer time (Segal, 1982). ACh did not affect the small after-hyperpolarization which occasionally follows a train of action potentials generated in response to a 0·5 s depolarizing current pulse (data not shown). The responses to ACh were antagonized by atropine (0·1 mM-solution in a microdrop) indicating that it is mediated by a muscarinic receptor (three cells tested, data not shown). Finally, the responses to ACh could be evoked in the presence of TTX (Fig. 17) and Cd^{2+} (Fig. 16), indicating that they are not mediated by voltage-dependent Na^+ or Ca^{2+} currents.

5-hydroxytryptamine (*5-HT*)

5-HT constitutes a significant inhibitory afferent to m.s. neurones (Assaf & Miller, 1978). In the hippocampus and elsewhere (Segal, 1980) 5-HT seems to cause an

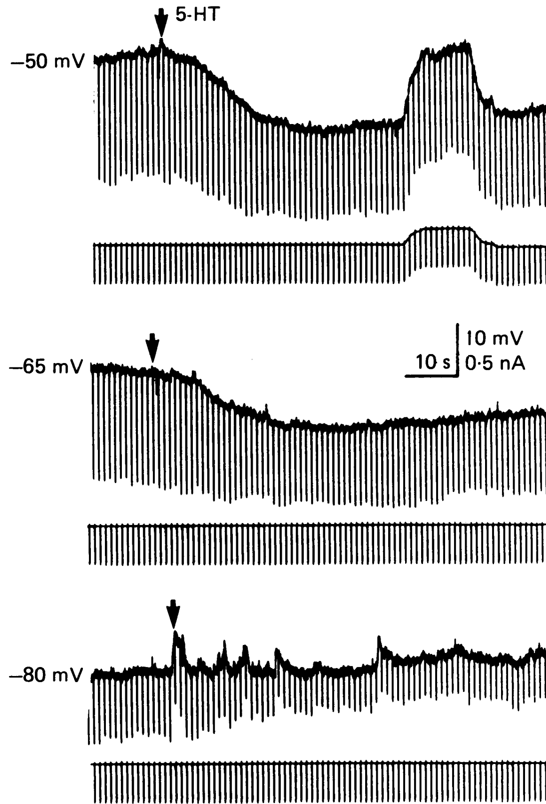

Fig. 18. 5-HT hyperpolarizes a m.s. neurone. The cell was depolarized to −50 mV (top trace), kept at rest (−65 mV, middle trace) and hyperpolarized to −80 mV (bottom) in the presence of TTX (1 μM) and Cd^{2+} (1 mM). 5-HT, applied in a microdrop containing 0·1 mM-solution at the arrowhead, hyperpolarized the cell by 14 mV (top) and by 9 mV (middle) but did not change the resting potential (bottom record) when the cell was already hyperpolarized. The response to 5-HT was associated with an increase in conductance; when the cell was depolarized back to pre-drug potential (top record) the conductance increase was still evident. KCl recording micropipette.

increase in K^+ conductance in a Ca^{2+}- and voltage-independent manner. 5-HT was applied to twelve m.s. neurones. In nine of these it produced a hyperpolarizing response associated with an increase in conductance (Fig. 18). The initial hyperpolarization was occasionally accompanied by a transient increase in discharges of s.p.s.p.s. The response to 5-HT reversed at about −80 mV, indicating that it involves primarily a K^+ current. It was present in cells treated with TTX and Cd^{2+}. Interestingly, the responses to 5-HT were far shorter in duration than those of hippocampal neurones measured in the same manner as seen with the response to ACh. A complete recovery from effects of 5-HT was seen 1–2 min after drug application.

DISCUSSION

The present experiments examined some physiological properties of m.s. neurones in an attempt to relate these to the unique behaviour of these neurones in the intact brain. The fact that the m.s. can drive hippocampal slow rhythmic activity lends further significance to the understanding of the mechanisms governing m.s. activity. Most of the m.s. neurones recorded in the slice either fired spontaneously or their resting potential was near the firing threshold. Some of these neurones fired repetitively during the passage of a constant depolarizing pulse and showed little accommodation. Repetitive firing of Ca^{2+} spikes could also be recorded in cells where voltage-dependent Na^+ channels were blocked by TTX. In the presence of TTX, Na^+ action potentials are blocked and communication between cells is nearly absent. It thus appears that the mechanism for generation of rhythmic activity is intrinsic to m.s. neurones.

Ca^{2+} currents probably play a dominant role in eliciting repetitive firing in m.s. neurones. The doublets seen in response to a short depolarizing current pulse are blocked by Ca^{2+} antagonists, indicating that Ca^{2+} is involved in generation of the doublets. Ca^{2+} currents also underlie the repetitive Ca^{2+} spikes seen in the presence of TTX and intracellular Cs^+. It appears that at resting potential a Ca^{2+} conductance is gradually activated and brings the cell near its Ca^{2+} spike firing level. The fact that it is not detected very well normally and can only be seen in Cs^+-loaded cells may indicate that it resides on remote dendrites.

The relative absence of several K^+ current species contributes to the generation of high discharge rates of action potentials in m.s. neurones. First is the minimal contribution of a transient outward current to the interspike interval in these neurones. Attempts to detect presence of a transient outward current were made by priming cells into hyperpolarized potentials followed by a depolarizing pulse sufficient to evoke action potentials. Such a paradigm uncovers a large transient outward rectification in other neurone types (Connor & Stevens, 1971; Llinas & Yarom, 1981; Segal, 1986) but not in m.s. neurones (data not shown). Second is the relatively minor presence of a Ca^{2+}-dependent K^+ current responsible for the after-hyperpolarization seen elsewhere (Wong & Prince, 1978; Cole & Nicoll, 1984). If indeed activation of an a.h.p. is responsible for accommodation it is then clearly reduced to a large extent in m.s. neurones.

While the m.s. seem to possess intrinsic mechanisms needed to generate rhythmic activity, two mechanisms need further explorations. First is the question of what triggers the m.s. into its rhythmic activity or, alternatively, releases it from such activity; secondly, how m.s. neurones are synchronized to generate a wave of activity in the hippocampus.

One possible source of control mechanism is the serotonin-containing mid-brain raphe. It projects an inhibitory pathway into the m.s. which can stop abruptly ongoing septal activity. Since most serotonin neurones of the rostral mid-brain have a similar behavioural repertoire, their concurrent activation might cause cessation of septal activity. On the other hand, a cholinergic input may promote synchronized septal activity through recurrent activation of septal neurones. If indeed the cholinergic innervation of the m.s. is at least partially of a local origin, it may serve

as a positive feed-back generator to escalate m.s. activity. Interestingly, both serotonin and ACh have short duration of action in the m.s. compared to their duration in the hippocampus, indicating that the m.s. can be activated or blocked rather rapidly by behavioural signals.

The present experiments described two types of neurones in the m.s.: spontaneous, high-resistance, and quiescent, low-resistance neurones. While it is implied that the former type corresponds to bursting septohippocampal neurones, many of which are cholinergic (Baisden *et al.* 1984), we have no firm evidence to support this assumption as yet. Experiments are currently under way in an attempt to double-label recorded m.s neurones and identify them as efferent (using retrograde tracing) and/or cholinergic (i.e. choline acetyltransferase immunoreactive) neurones.

Even if the hypothesis is confirmed, one has still to be cautious in extrapolating the mechanism of septal bursting in a slice to that found in the intact brain. Septal rhythmicity in the brain has a unique pattern (Segal, 1976) not seen in the slice. The different recording conditions, ion composition, temperature, blood flow etc. can contribute to this difference.

The synchronizing mechanism is still unclear. A large proportion of LY dye coupling was found in the m.s., especially among small cells (Fig. 1). This does not imply that the cells are electrically coupled since dye coupling is a frequent finding in slice preparations and more direct experiments are needed to test this possibility. However, if electrical coupling does exist, it may constitute the anatomical basis for synchronized activity.

REFERENCES

ANDERSEN, P., DINGLEDINE, R., GJERSTAD, L., LANGMOEN, I. A. & MOSFELDT LAURSEN, A. M. (1980). Two different responses of hippocampal pyramidal cells to application of gamma-aminobutyric acid. *Journal of Physiology* **305**, 279–296.

APOSTOL, G. & CREUTZFELDT, O. D. (1974). Cross correlation between the activity of septal units and hippocampal EEG during arousal. *Brain Research* **67**, 65–75.

ASSAF, S. Y. & MILLER, J. J. (1978). The role of a raphe serotonin system in the control of septal unit activity and hippocampal desynchronization. *Neuroscience* **3**, 539–550.

BAISDEN, R. H., WOODRUFF, M. L. & HOOVER, D. B. (1984). Cholinergic and non cholinergic septo hippocampal projections: a double label horseradish peroxidase acetylcholinesterase study in the rabbit. *Brain Research* **290**, 146–151.

BUTCHER, L. L. (1978). Recent advances in histochemical techniques for the study of recent cholinergic mechanism. In *Cholinergic Mechanisms and Psychopharmacology*, ed. JENDEN, D. J., pp. 93–124. New York: Plenum.

BUZSAKI, G., LEUNG, L. W. S. & VANDERWOLF, C. H. (1984). Cellular bases of hippocampal EEG in the behaving rat. *Brain Research Reviews* **6**, 139–171.

COLE, E. & NICOLL, R. A. (1984). Characterization of a slow cholinergic post-synaptic potential recorded *in vitro* from rat hippocampal pyramidal cells. *Journal of Physiology* **352**, 173–188.

CONNOR, J. A. & STEVENS, C. F. (1971). Voltage clamp studies of a transient outward membrane current in gastropod neural somata. *Journal of Physiology* **213**, 21–30.

DUTAR, P., LAMOUR, Y. & JOBERT, A. (1985). Septohippocampal neurons in the rat: an *in vivo* intracellular study. *Brain Research* **340**, 135–142.

FRENCH, C. R. & GAGE, P. W. (1985). A threshold sodium current in pyramidal cells in rat hippocampus. *Neuroscience Letters* **56**, 289–293.

GRAY, J. A. & MCNAUGHTON, N. (1983). Comparison between the behavioral effects of septal and hippocampal lesions: a review. *Neuroscience and Biobehavioral Reviews* **7**, 119–188.

GOGOLAK, G., STUMP, C. H., PETSCHE, H. & STERC, F. (1968). The firing patterns of septal neurons and the form of hippocampal theta wave. *Brain Research* **7**, 201–207.

HAGIWARA, S. (1973). Calcium spikes. *Advances in Biophysics* **4**, 71–102.

HALLIWELL, J. V. & ADAMS, P. R. (1982). Voltage clamp analysis of muscarinic excitation in hippocampal neurons. *Brain Research* **250**, 71–92.

JAHNSEN, H. & LLINAS, R. (1984). Ionic basis for the electroresponsiveness and oscillatory properties of guinea-pig thalamic neurones *in vitro*. *Journal of Physiology* **349**, 227–247.

KANDEL, E. R. & SPENCER, W. A. (1961). Electrophysiology of hippocampal neurons II: after potentials and repetitive firing. *Journal of Neurophysiology* **24**, 243–259.

LAMOUR, Y., DUTAR, P. & JOBERT, A. (1984). Septo-hippocampal and other medial-septum diagonal band neurons: electrophysiological and pharmacological properties. *Brain Research* **309**, 227–239.

LLINAS, R. & YAROM, Y. (1981). Electrophysiology of mammalian inferior olivary neurones *in vitro*. Different types of voltage-dependent ionic conductances. *Journal of Physiology* **315**, 549–567.

MACADAR, O., ROIG, J. A., MONTI, J. M. & BUDELLI, R. (1970). The functional relationship between septal and hippocampal unit activity and hippocampal theta rhythm. *Physiology and Behaviour* **5**, 1443–1449.

MCLENNAN, H. & MILLER, J. J. (1974). The hippocampal control of neuronal discharges in the septum of the rat. *Journal of Physiology* **237**, 607–624.

PETSCHE, H., STUMPF, C. H. & GOGOLAK, G. (1962). The significance of the rabbit septum as a relay station between the midbrain and the hippocampus I. Control of hippocampal arousal activity by the septum cells. *Electroencephalography and Clinical Neurophysiology* **14**, 202–211.

PURPURA, D. P., PRELEVIC, S. & SANTINI, M. (1968). Hyperpolarizing increase in membrane conductance in hippocampal neurons. *Brain Research* **7**, 310–312.

SEGAL, M. (1973). Flow of conditioned responses in the limbic telencephalic system of the rat. *Journal of Neurophysiology* **36**, 840–854.

SEGAL, M. (1976). Brainstem afferents to the rat medial septum. *Journal of Physiology* **261**, 617–631.

SEGAL, M. (1980). The action of serotonin in the rat hippocampal slice preparation. *Journal of Physiology* **303**, 423–439.

SEGAL, M. (1982). Multiple actions of acetylcholine at a muscarinic receptor in rat hippocampal slices. *Brain Research* **246**, 77–87.

SEGAL, M. (1986). A potent transient outward current regulates excitability of dorsal raphe neurons. *Brain Research* **359**, 347–350.

SEIFERT, W. (1983). *Neurobiology of Hippocampus*, ed. SEIFERT, W. London: Academic Press.

STAFSTROM, C. E., SCHWINDT, P. C., CHUBB, M. C. & CRILL, W. C. (1985). Properties of persistent sodium conductance and calcium conductance of layer V neurons from cat sensorimotor cortex *in-vitro*. *Journal of Neurophysiology* **53**, 153–170.

SWANSON, L. W. & COWAN, W. M. (1979). The connections of the septal region in the rat. *Journal of Comparative Neurology* **186**, 621–656.

VANDERMAELEN, C. P. & AGHAJANIAN, G. K. (1983). Electrophysiological and pharmacological characterization of serotonergic dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices. *Brain Research* **289**, 109–119.

VINOGRADOVA, O. S., BRAZHNIK, E. S., KARANOV, A. M. & ZHADINA, S. D. (1980). Neuronal activity of the septum following various types of deafferentation. *Brain Research* **187**, 353–368.

WAINER, B. H., LEVEY, A. I., RYE, D. B., MESULAM, M. M. & MUFSON, E. J. (1985). Cholinergic and non cholinergic septohippocampal pathways. *Neuroscience Letters* **54**, 45–52.

WONG, R. K. S. & PRINCE, D. A. (1978). Participation of calcium spikes during intrinsic burst firing in hippocampal neurons. *Brain Research* **159**, 385–390.