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IONIC BASIS OF THE DIFFERENTIAL NEURONAL ACTIVITY OF GUINEA-PIG SEPTAL NUCLEUS STUDIED *IN VITRO*

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SUMMARY

1. The electrical properties and ionic conductances of septal neurones were studied by intracellular recording in an *in vitro* slice preparation. Within the total number of cells recorded ($n = 150$) we identified three electrophysiological cell types, each one of them located in a separate septal region. Dorsolateral septal neurones comprised 60% of the cells, intermediate septal neurones 10%, and medial septal neurones 30%.

2. Passive electrical constants of dorsolateral, intermediate and medial septal neurones were, respectively: resting potential ($-60{\cdot}2 \pm 4{\cdot}8$, $-59{\cdot}8 \pm 3{\cdot}3$ and $-56 \pm 4{\cdot}3$ mV); input resistance ($82{\cdot}5 \pm 17$, 63 ± 16 and 83 ± 18 MΩ) and membrane time constant ($18{\cdot}5 \pm 7{\cdot}3$, $14{\cdot}2 \pm 6{\cdot}8$ and $10{\cdot}7 \pm 3{\cdot}4$ ms).

3. Direct activation of dorsolateral septal neurones by current injection below 0·2 nA triggered repetitive firing of fast action potentials. Larger current pulses elicited a characteristic response consisting of an initial fast action potential followed by a train of slow spikes. An after-hyperpolarization followed termination of the pulse and the characteristic response.

4. In dorsolateral septal neurones tetrodotoxin (TTX) abolished the fast action potentials. The slow spikes and the after-hyperpolarization disappeared in presence of Co^{2+} or after brief removal of external Ca^{2+}. This suggests that the characteristic response is mediated by Ca^{2+} and the after-hyperpolarization by a Ca^{2+}-dependent K^+ conductance.

5. The firing pattern of intermediate septal neurones activated from the resting potential spontaneously measured in the cells was similar to that of dorsolateral septal neurones; but direct activation from a hyperpolarized membrane potential evoked in intermediate septal cells a bursting response due to the generation of a low-threshold spike. The low-threshold spike was TTX-resistant but abolished by Co^{2+} and reached a maximal amplitude after hyperpolarization to -75 mV lasting for 100–150 ms. These results suggest the existence in intermediate septal neurones of a low-threshold Ca^{2+} conductance inactivated at the resting potential and de-inactivated by hyperpolarization.

6. Depolarization of medial septal neurones by current pulses of amplitude greater than 0·2–0·3 nA elicited a typical burst of two to six action potentials. The bursts lasted for 20–50 ms and were followed by a marked after-hyperpolarization. In the

presence of TTX bursts of slow spikes were generated. These Ca^{2+}-dependent slow spikes were abolished by Co^{2+} and increased in amplitude by 4-aminopyridine (4-AP) and Ba^{2+}. The after-hyperpolarization was unaffected by TTX and 4-AP but depressed by Ba^{2+} and Co^{2+}.

7. Medial septal neurones displayed a characteristic persistent hyperpolarization at the termination of negative pulses of over 10–20 mV lasting more than 5–10 ms, which was accompanied by a decrease of input resistance. This hyperpolarization was unaffected by TTX and 4-AP but was markedly reduced by Co^{2+} and the brief removal of external Ca^{2+}. This response, that may be due to a transient Ca^{2+}- and voltage-dependent K^+ current, was probably the membrane mechanism that prevented repetitive firing.

8. These results demonstrate the existence of striking differences in the electrical properties of cells located in separate septal regions. These intrinsic membrane properties appear to place limits on the excitability of septal cells that in the case of medial septal neurones might be the basis for the generation of the theta rhythm.

INTRODUCTION

During the last three decades the septal nuclei have attracted the attention of several investigators due to their close anatomical and functional relations with the hippocampus and other areas of the limbic system, and the correlation between single septal cell activity and hippocampal EEG has been examined in detail (Green & Arduini, 1954; Brücke, Petsche, Pillat & Deisenhammer, 1959; Apostol & Creutzfeldt, 1974; McLennan & Miller, 1976; De France, Yoshihara & Chronister, 1976; Vinogradova, Brazhnik, Karanov & Zhadina, 1980; Lamour, Dutar & Jobert, 1984; Dutar, Lamour & Jobert, 1985). However, virtually nothing is known about the electrophysiological characteristics and membrane ionic conductances of individual neurones located in the septal region. It seemed to us therefore of special interest to elucidate whether the subdivision of the septum into various regions or nuclei based on anatomical and functional studies (Raisman, 1966; McLennan & Miller, 1974; De France *et al.* 1976; Meibach & Siegel, 1977) also occurred at the level of the intrinsic membrane characteristics of the single septal cell. Some preliminary intracellular recordings have been performed in the rat (Stevens, Gallagher & Shinnick-Gallagher, 1984; Joëls, Twery, Shinnick-Gallagher and Gallagher, 1986) and in the guinea-pig (López-Barneo, Alvarez de Toledo & Yarom, 1985) lateral septum but a complete analysis of the electrical properties of neurones located in this nucleus and a comparison with the properties of the cells located in the other septal regions was not available. These questions are addressed in this paper where the whole septal area has been explored. Some of the data presented here have been published in abstract form (Alvarez de Toledo & López-Barneo, 1986).

METHODS

The techniques used for tissue preparation and for intracellular recording have been described extensively (Llinás & Sugimori, 1980; Dingledine, 1984), and therefore only a brief account of our experimental arrangement is given. Adult guinea-pigs were decapitated and a piece of the brain containing the septal region was isolated and placed in the stage of a vibratome where six coronal slices, 500 μm thick, containing part of the septal nuclei, were obtained. These slices comprised the

brain area between co-ordinates 13 and 10·6 anterior according to the atlas of Luparello (1967). After sectioning, the slices were incubated in separate vials at room temperature with oxygenated standard solution for at least 1 h. During the experimental procedure a slice was transferred to the recording chamber where it was continuously superfused with the standard solution and maintained at 37 °C. The standard solution had the following composition (mM): NaCl, 124; KCl, 4·9; $MgSO_4$, 1·3; NaH_2PO_4, 1·2; $CaCl_2$, 2·4; $NaHCO_3$, 25; glucose, 10. Tetrodotoxin (TTX) was added to block Na^+ conductance and tetraethylammonium chloride (TEA^+) and 4-aminopyridine (4-AP) to block different types of K^+ channels. In experiments where the effects of divalent cations were tested, bicarbonate, sulphate and phosphate were omitted from the standard solution and Ca^{2+} was substituted by Ba^{2+} or Co^{2+}.

Intracellular recordings were obtained from septal neurones using glass microelectrodes filled with 3 M-potassium acetate with a DC resistance of 50–80 MΩ. The microelectrodes were connected to the headstage of an intracellular recording amplifier built in our laboratory (López-Barneo & Armstrong, 1983). Impalements were facilitated by a brief (about 5 ms) and large electronic oscillation of the headstage amplifier. Quantitative analysis of the data was performed off-line with the aid of a microcomputer (IBM, model PC-XT, U.S.A.). For this purpose the voltage and current analog signals were digitized by the analog-to-digital converter (10 bits) of a two-channel digital oscilloscope (Tektronix, model 5200, U.S.A.) interfaced to the computer by an IEEE-488 card (National Instruments, U.S.A.).

RESULTS

The data presented in this paper were obtained from 150 neurones located in different areas of the septum. The cells selected for this study were held for at least 15 min with a resting membrane potential more negative than −50 mV and stable electrical properties.

Location of cells recorded

The distribution of a representative sample of cells recorded in the different septal areas is shown in Fig. 1. Within the total number of cells 60% were dorsolateral septal neurones, 30% medial septal neurones and 10% intermediate septal neurones. These percentages parallel the distribution of neurones seen in cytoarchytectonic studies (Cajal, 1911) but they may also reflect differences in cell size and in the viability of each neuronal type after the slicing procedure.

Dorsolateral septal neurones

Electrical parameters and firing pattern. The average electrical parameters of a representative sample of dorsolateral septal neurones are shown in Table 1. About 70% of the cells were silent at rest. The average resting potential was $-60{\cdot}2 \pm 4{\cdot}8$ mV (mean ± S.D.). The remaining 30% fired at the resting potential at frequencies of 2–10 Hz. The input resistance of dorsolateral septal neurones was $82{\cdot}5 \pm 17$ MΩ and the membrane time constant $18{\cdot}5 \pm 7{\cdot}3$ ms (Table 1).

Direct activation of dorsolateral septal neurones by current injections below 0·2 nA elicited a train of fast action potentials (Fig. 2*A*) but as the amplitude of the pulse was increased these cells displayed a characteristic firing pattern which consisted of an initial fast action potential followed by a train of smaller and slower spikes that lasted for the whole duration of the pulse (Fig. 2*B*). Termination of the pulse and spike train was followed by an after-hyperpolarization whose duration and amplitude increased with the strength of the pulse. (Fig. 2*A* and *B*). The application of negative current pulses revealed the presence of anomalous rectification in 90% of dorsolateral septal neurones.

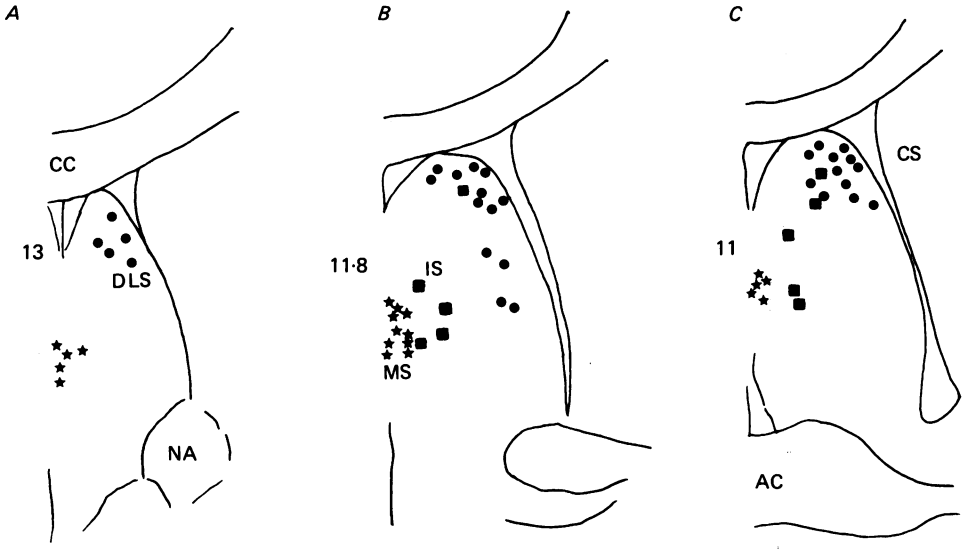

Fig. 1. Location of sixty septal neurones, the electrical parameters of which are shown in Table 1. Each neuronal class identified in the septum is represented by a different symbol (●, dorsolateral septal neurones; ■, intermediate septal neurones; ★, medial septal neurones). The reconstruction of the coronal sections is based on the atlas of Luparello (1967). Numbers on the left of each section correspond to the distance in millimetres anterior to the interaural stereotaxic plane. Abbreviations: CC, corpus callosum; CS, corpus striatum; NA, nucleus accumbens; AC, anterior commissure; DLS, dorsolateral septum; IS, intermediate septum; MS, medial septum.

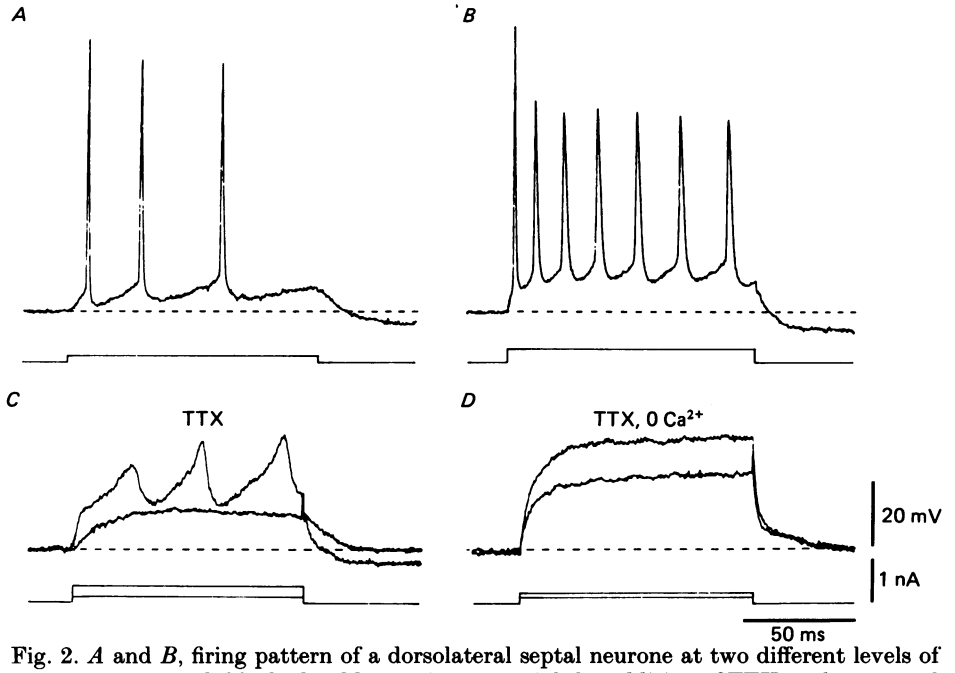

Fig. 2. *A* and *B*, firing pattern of a dorsolateral septal neurone at two different levels of current injection. *C*, blockade of fast action potentials by addition of TTX to the external solution and generation of slow, Ca^{2+} spikes by the largest depolarizing pulse. Note in records *A–C* the after-hyperpolarization following the current pulses. *D*, both the Ca^{2+} spikes and the after-hyperpolarization disappeared after removal of external Ca^{2+}. The resting potential, −57 mV, is indicated by the dashed line.

TABLE 1. Electrophysiological parameters of cells shown in Fig. 1

					Fast action potential			
	Number of measurements	V_{rest} (mV)	IR (MΩ)	Time constant (ms)	V_{spike} (mV)	Thresh. (mV)	dV/dt_r (V/s)	dV/dt_f (V/s)
Dorsolateral neurones	30	60·2±4·8	82·5±17	18·5±7·3	76·8±7	12±4·6	177±34	100±26
Intermediate neurones	10	59·8±3·3	63±16	14·2±6·8	77·4±9	9·8±1·4	175±24	121±32
Medial neurones	20	56±4·3	83±18	10·7±3·4	78·9±10	11±3·3	186±35	124±32

All values are mean±S.D. Abbreviations: V_{rest}, resting potential; IR, input resistance; V_{spike}, action potential amplitude measured from the resting potential; Thresh., action potential threshold measured from the resting potential: dV/dt_r, maximum rate of rise of action potential upstroke; dV/dt_f, maximum rate of falling of action potential downstroke. Input resistance was measured from the slope of current–voltage curves obtained by injecting small (less than 0·3 nA) hyperpolarizing current pulses. The time constant was calculated as the time at which a hyperpolarization elicited by a square-current step of small amplitude reached 63% (1–1/e) of its final value.

Ca^{2+}-dependent spikes. Although the addition of TTX to the external solution (Blankenship & Kuno, 1968) abolished the fast action potentials, moderate depolarizations elicited at a higher threshold slow, TTX-resistant spikes that were generated repetitively (Fig. 2*C*). These spikes were Ca^{2+}-dependent since they were blocked by Co^{2+} (Kohlhardt, Bauer, Krause & Fleckenstein, 1973) or following the

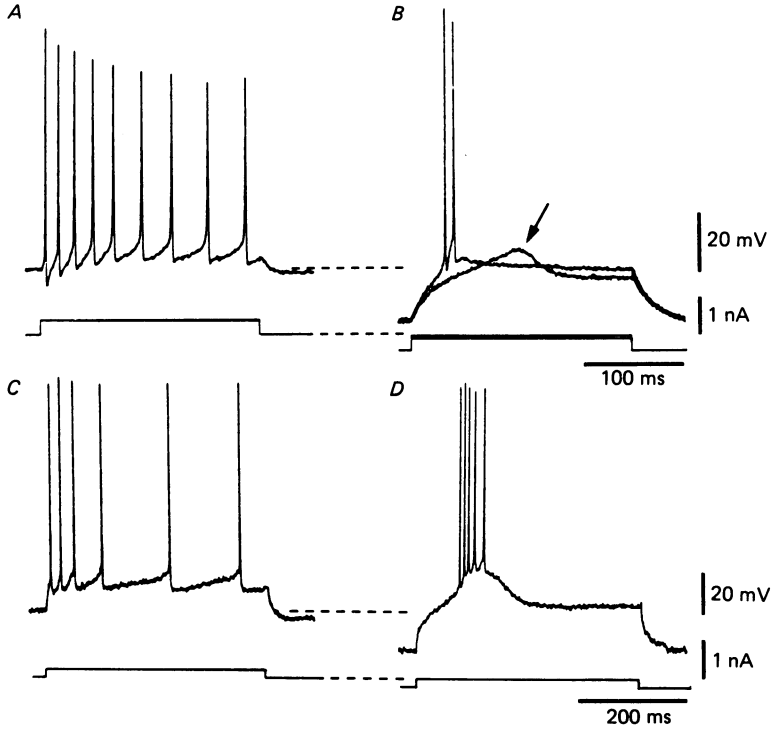

Fig. 3. Tonic-to-burst firing switching in intermediate septal neurones. Activation of the cell at the resting potential evoked a tonic discharge of action potentials (*A*). When the cell was depolarized from a membrane potential level 22 mV more negative than the resting value it fired a burst of spikes due to the generation of a low-threshold, slow spike (arrow) the generating mechanism of which was de-inactivated by the maintained hyperpolarization (*B*). Resting potential, −55 mV. Records *C* and *D* illustrate the tonic and bursting firing modes in another cell. Resting potential, −64 mV. Resting potential and current levels are indicated by the dashed lines.

brief removal of external Ca^{2+} (Fig. 2*D*) Fig. 2*C* and *D* also show that the after-hyperpolarization seen in dorsolateral septal neurones at the termination of depolarizing pulses and following the train of Ca^{2+} spikes was still generated in the presence of TTX and was abolished in the absence of external Ca^{2+}. This observation suggests that a Ca^{2+}-activated K^+ conductance may be responsible for the after-hyperpolarization. Transient removal of Ca^{2+} did not appreciably alter the resting potential of the cells but increased the amplitude of passive depolarizing responses by almost 100% (compare traces in Fig. 2*C* and *D*).

Intermediate septal neurones

Electrical parameters and firing pattern. The electrical parameters of intermediate septal neurones are shown in Table 1. These parameters are not significantly different from those of dorsolateral septal neurones with the exception of the somewhat smaller input resistance. The firing pattern of intermediate septal neurones activated

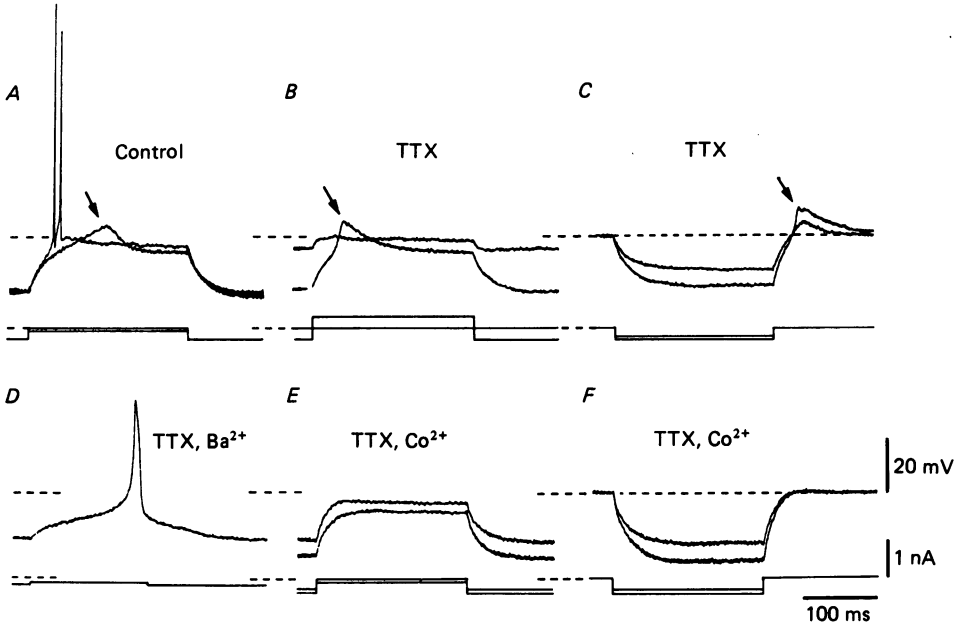

Fig. 4. Ionic dependence of the low-threshold spike of intermediate septal neurones. *A*, bursting response in a cell activated by a current pulse delivered on top of a hyperpolarization of 20 mV. The low-threshold spike is indicated by an arrow. *B*, the fast action potentials were abolished by TTX but the slow spike could be generated when the cell was activated from a hyperpolarized membrane potential level. *C*, at the cell resting potential the low-threshold spike also appeared at the break of hyperpolarizing pulses. *D*, replacement of Ca^{2+} by Ba^{2+} increased the amplitude of the low-threshold spike, which totally disappeared after adding Co^{2+} (2 mM) to the external solution (*E* and *F*). Resting potential (−55 mV) and current levels are indicated by the dashed lines.

by current pulses of low amplitude when the cells were held at the resting potential resembled that of dorsolateral septal neurones and 65% of the cells showed anomalous rectification. Intermediate septal neurones were unique in that depolarizing pulses applied to the hyperpolarized cell generated a slow depolarizing potential which could evoke a burst of fast action potentials. This electrical characteristic is illustrated in Fig. 3*A–D* with recordings obtained from two different cells.

Low-threshold Ca^{2+} spikes. The ionic mechanisms responsible for the bursting response of intermediate septal neurones are analysed in Fig. 4. Fast action potentials generated in the control situation (*A*) were abolished by addition of TTX to the external solution, while the low-threshold spike remained unaltered (*B*). In *C* a brief hyperpolarization is also shown to evoke a large rebound depolarization.

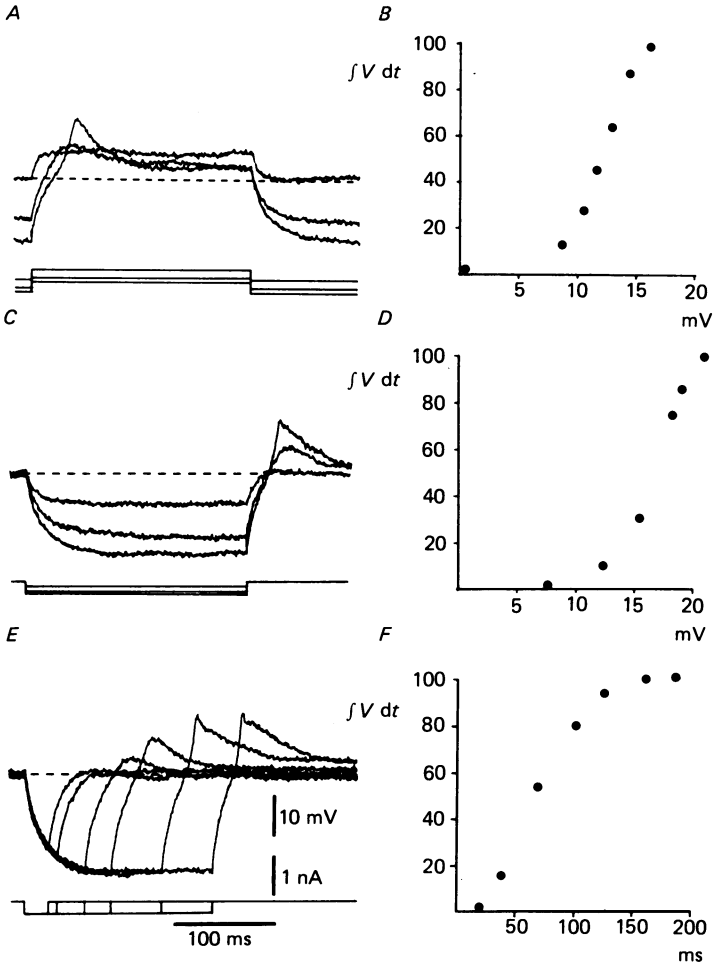

Fig. 5. Dependence of the low-threshold Ca^{2+} spike on the amplitude and duration of the preceding membrane hyperpolarization. *A* and *B*, low-threshold spike elicited by depolarizing current pulses delivered on top of a hyperpolarization of variable amplitude. The normalized plot illustrates the time integral of the spike (ordinate) as a function of the amplitude of the hyperpolarization (abscissa). *C* and *D*, low-threshold spike generated at the break of hyperpolarizing pulses of variable amplitude. The relationship between the time integral of the active voltage response (ordinate) and the amplitude of the preceding hyperpolarization (abscissa) is shown in the normalized plot. *E* and *F*, low-threshold spike triggered at the break of negative pulses of constant amplitude but variable duration. The plot shows the increase of the time integral of the voltage response (ordinate) as the duration of the hyperpolarizing pulse (abscissa) is progressively made longer. In all records Na^+ conductance was blocked by TTX. The cell resting potential (−55 mV) is indicated by the dashed lines.

Replacement of Ca^{2+} by Ba^{2+}, which has a greater permeability than Ca^{2+} through Ca^{2+} channels (Hagiwara, Fukuda & Eaton, 1974), increased the amplitude of the slow spike, although the threshold did not change appreciably (Fig. 4*D*). The increase in amplitude of the low-threshold spike may also reflect in part a failure of the entry of Ba^{2+} as opposed to Ca^{2+} to initiate an increase in K^+ conductance. The

spike generated either by membrane depolarization or at the break of negative current pulses disappeared when Co^{2+} was introduced in the external solution (Fig. 4*E* and *F*). These results suggest that the low-threshold spike was due to the activation of a Ca^{2+} conductance.

The voltage and the time dependence of the low-threshold spike in the presence of TTX was further examined, as shown in Fig. 5 *A* and *B* which illustrates the

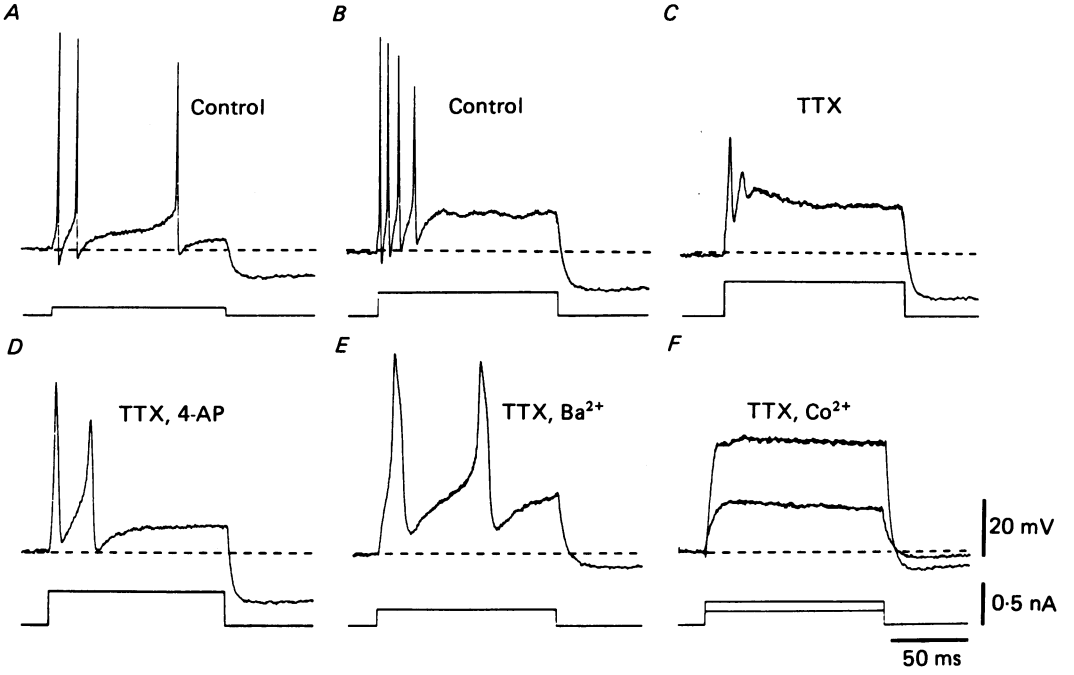

Fig. 6. *A* and *B*, firing pattern of a medial septal neurone activated by two different levels of current injection (control traces). In presence of TTX (*C*) fast action potentials disappeared but a burst of slow Ca^{2+} spikes and the after-hyperpolarization following the pulse were present. External application of 4-aminopyridine (4-AP, 3 mM) increased the amplitude of the Ca^{2+} spikes without having an affect on the after-hyperpolarization (*D*). On replacement of external Ca^{2+} by Ba^{2+} the slow spikes had a larger amplitude but the after-hyperpolarization was reduced (*E*). These spikes were abolished by addition of Co^{2+} (2 mM, *F*). In all cases the resting potential was maintained at the control value of −55 mV.

progressive increase of the low-threshold spike as the membrane was progressively hyperpolarized. Similar results were obtained when the low-threshold spike was generated at the termination of hyperpolarizing pulses (Fig. 5*C* and *D*). In this cell the low-threshold spike-generating mechanism is inactivated at the cell resting potential (−55 mV) and de-inactivates at 10–15 mV hyperpolarization. The generation of a full spike required a hyperpolarization of over 20 mV. Figure 5*E* illustrates the gradual appearance of the low-threshold spike as the duration of the negative current pulse increased. The normalized plot (Fig. 5*F*) shows that with a hyperpolarization of about 25 mV full deinactivation of the low-threshold spike-generating conductance was attained after 100–150 ms.

Medial septal neurones

Electrical parameters and firing pattern. The electrical parameters of medial septal neurones (Table 1) were similar to those of the other septal neurones, with the exception of significantly different average resting potential ($-56 \pm 4{\cdot}3$ mV) and membrane time constant ($10{\cdot}7 \pm 3{\cdot}4$). About 25% of medial septal neurones recorded were spontaneously active and discharged tonically at frequencies of 3–6 Hz.

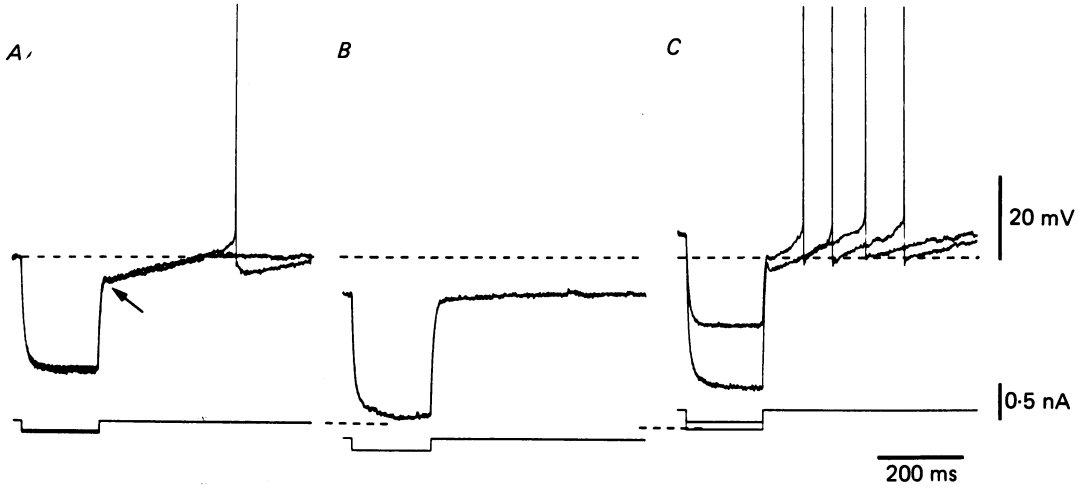

Fig. 7. Membrane voltage response of medial septal neurones to hyperpolarizing current injection. *A*, passive hyperpolarization and slow return to the resting potential at the termination of the current pulse. The arrow indicates the break in the discharge of membrane capacitance and the onset of a delayed hyperpolarization lasting for 200–500 ms. An action potential was triggered after this membrane response, due to the generation of a small rebound depolarization. When the cell was held at a membrane potential about 8 mV more negative than the resting value, the delayed return to baseline was not observed (*B*); it was, however, enhanced by depolarization (*C*). In this last situation spontaneous firing of the cell partially masked the delayed hyperpolarization. The resting potential of the cell, −55 mV, and the zero current level are indicated by the dashed lines.

A particular electrophysiological feature of medial septal neurones was their tendency to fire a burst of action potentials when activated by current pulses of an amplitude over 0·2 nA. Regardless of the duration of the current pulses the bursts lasted for 20–50 ms and comprised two to six action potentials that progressively decreased in amplitude and increased in duration. After the burst the cells were silent for the rest of the pulse (Fig. 6*A* and *B*). At the termination of depolarizing pulses medial septal neurones generated a pronounced after-hyperpolarization whose amplitude and duration increased with the strength of the preceding depolarization. Although TTX blocked the fast action potentials, large depolarizations could still elicit bursts of slow and small spikes (Fig. 6*C*). The slow spikes increased in amplitude and appeared at a lower threshold when 4-AP, that blocks at least one type of K^+ channel in central neurones (Gustafsson, Galvan, Grafe & Wigström, 1982), was added to the external solution (Fig. 6*D*). These spikes were most probably

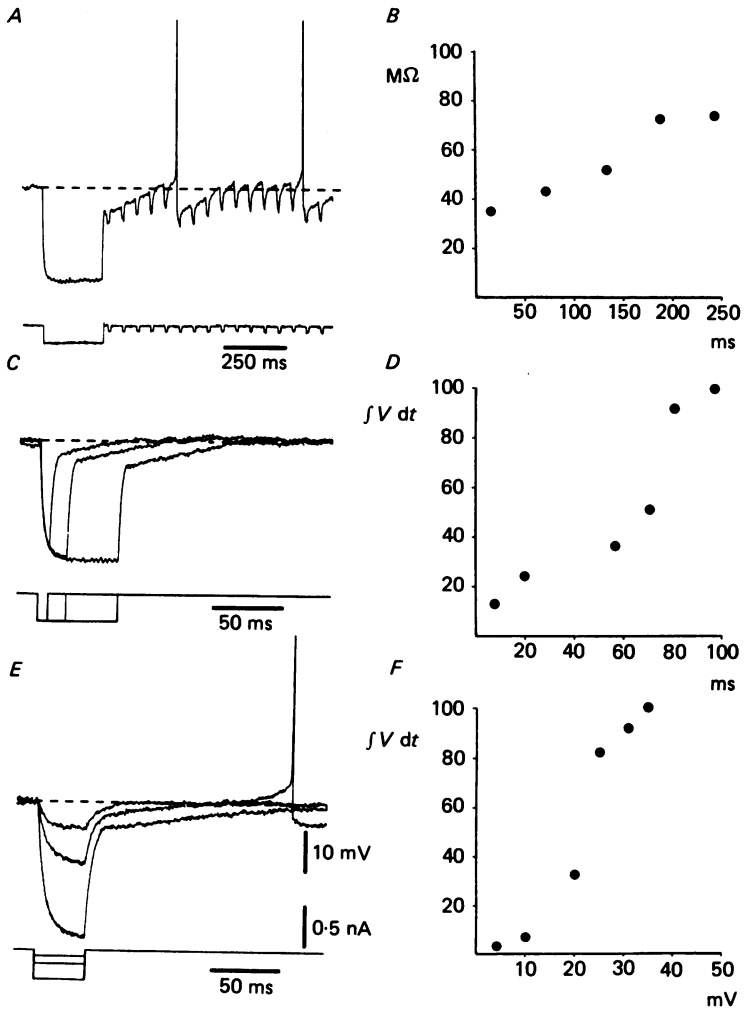

Fig. 8. *A* and *B*, changes of input resistance during the delayed return to baseline. Input resistance was monitored with 0·07 nA negative current pulses delivered at a frequency of 20 Hz. Note the gradual increase of input resistance as the voltage signal approached the resting level, and the changes of input resistance during the hyperpolarizing after-potentials following action potentials which were spontaneously generated by the cell. Spikes have been partially truncated to facilitate the composition of the Figure. Resting potential, −62 mV. *C–F*, dependence of the slow return to baseline on the duration and amplitude of the preceding hyperpolarization. Superimposed voltage records illustrate the augmentation of the slow return to resting as the duration (*C*) and the amplitude (*E*) of the current pulse increased. These effects were quantified by measuring the time integral of the delayed hyperpolarization, as shown in the normalized plots *D* and *F*. Integration limits were the inflexion between the fast and slow components and the time at which the voltage signal crossed the baseline. Resting potential, −55 mV, is indicated by the dashed line.

due to activation of a Ca^{2+} current since they were enhanced, and appeared tonically, after replacement of Ca^{2+} by Ba^{2+} (Fig. 6*E*) and were abolished after addition of Co^{2+} (Fig. 6*F*). Figures 6*D–F* also show that the after-hyperpolarization was unaffected by 4-AP but was depressed in the presence of Ba^{2+} and Co^{2+}.

Delayed return to the resting potential after membrane hyperpolarization. A distinct electrophysiological property of all medial septal neurones, but not of dorsolateral

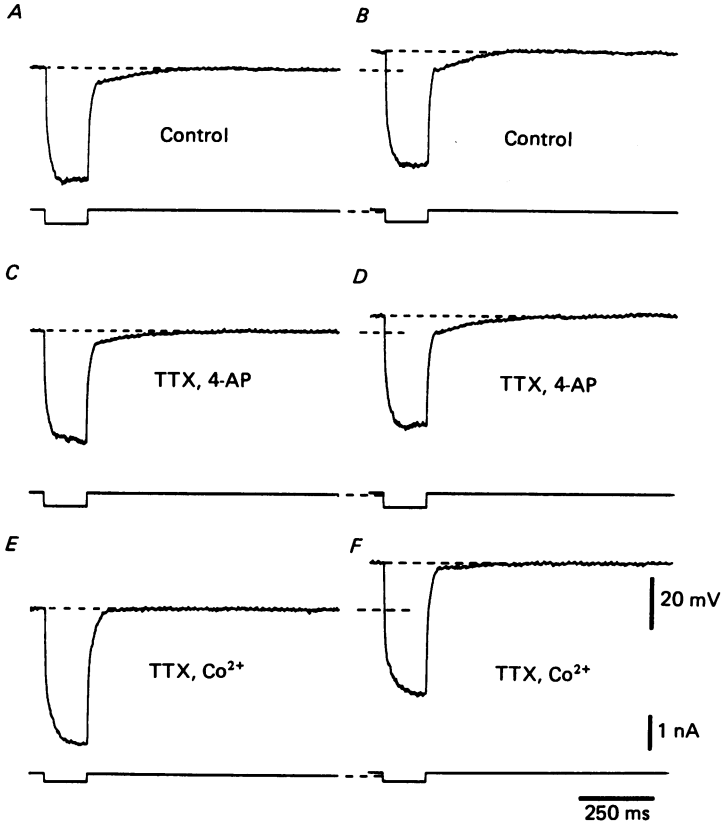

Fig. 9. Transient K^+ conductance of medial septal neurones. *A* and *B*, control recordings in the standard solution at two membrane potential levels, illustrating the slow return to baseline and its enhancement by depolarization. External application of 4-aminopyridine (4-AP, 3 mM) did not have a substantial effect on this subthreshold membrane voltage response (*C* and *D*); however it was abolished when external Ca^{2+} was changed for Co^{2+} (*E* and *F*). Resting potential in *C* and *E* was maintained at the same value as in *A* (−55 mV).

septal neurones and only seen in a single intermediate septal neurone, was a delayed return to the resting membrane potential after the termination of hyperpolarizing pulses. This phenomenon is illustrated in Fig. 7*A* where the return to the resting potential at the termination of the pulse had two clear components. The first, fast component, was due to the discharge of membrane capacitance and reached a level 6–7 mV below the resting potential of the cell. At this point there was a marked inflexion in the voltage signal (indicated by an arrow in the Figure) followed by a

second, smooth, slow component, that lasted for several tens of milliseconds. This biphasic return to the resting potential was not observed when the current pulses were applied while the cell was hyperpolarized by 8–10 mV (Fig. 7*B*). By contrast, depolarization (Fig. 7*C*) enhanced the inflexion between the fast and the slow components. In some medial septal neurones the slow return to baseline was followed by a small rebound depolarization large enough to reach the action potential threshold. The dependence of this response on the membrane potential suggests that it was due, as in other central neurones (Gustafsson *et al.* 1982; Llinás, 1984), to the activation of a transient K^+ current.

The delayed return to the resting potential was an active membrane electrical phenomenon paralleled by changes in the input resistance of the cells (Fig. 8*A* and *B*). This response was also dependent on the duration and amplitude of the preceding hyperpolarization as shown in Fig. 8 *C–F*. These results suggest that the membrane conductance generating the slow return to baseline is partially inactivated at the resting potential and de-inactivates by hyperpolarization of over 10–20 mV lasting more than 5–10 ms.

Figure 9 illustrates the effect of several blockers of transient K^+ current channels on the delayed return to the resting potential. Control recordings are shown in traces *A* and *B*. Neither TTX nor 4-AP had an appreciable effect on the transient response (traces *C* and *D*); however after 4-AP wash-out it was totally blocked by addition of Co^{2+} to the external solution (traces *E* and *F*).

DISCUSSION

Intracellular recordings from *in vitro* septal slices presented in this paper show striking differences in the electrical properties of neurones located in separate regions of the septum. Each cell type had a characteristic firing pattern and possessed particular membrane ionic conductances which might influence their integrative properties. This biophysical specialization within the septum corresponds quite well with its cytoarchitectonic organization (Cajal, 1911; Andy & Stephan, 1961), its afferent and efferent connections (Raisman, 1966; Meibach & Siegel, 1977) and the functional separation between the different septal areas (McLennan & Miller, 1974, 1976; De France *et al.* 1976; Lebrum & Poulain, 1983; Lamour *et al.* 1984). The electrical parameters of the three cell types identified in the septum were, although with small differences, somewhat similar and compatible with values measured in other CNS neurones (Brown, Fricke & Perkel, 1981; Connors, Gutnick & Prince, 1982; Jahnsen & Llinás, 1984).

The most characteristic electrical property of dorsolateral septal neurones is a transition from fast to slow spike firing and the restriction of their firing frequency. This unusual behaviour is not comparable with that of other central neurones recorded *in vitro* which show a tonic firing of fast action potentials in a broad range of depolarizing current injection (Connors *et al.* 1982; López-Barneo & Llinás, 1983; Jahnsen & Llinás, 1984). The limited excitability of dorsolateral septal neurones was due to the activation of a Ca^{2+} conductance, partial inactivation of Na^+ conductance, and probably, the fast onset of a Ca^{2+}-activated K^+ current. The blockade of this last conductance was probably responsible for the increase of passive

depolarizing responses observed in presence of Co^{2+} or by removal of external Ca^{2+} (see Fig. 2). Calcium-dependent spikes recorded in dorsolateral septal neurones are similar to the high-threshold Ca^{2+} spikes observed in almost every CNS neurone recorded *in vitro* which it is believed are generated in the dendrites (Schwartzkroin & Slawsky, 1977; Crill & Schwindt, 1983; Llinás, 1984).

Intermediate septal neurones differed from those recorded in the other septal areas in their ability to switch from a tonic to a burst firing mode when they were activated from membrane potentials more negative than −75 mV. This response was generated by a low-threshold Ca^{2+} current inactivated at the resting potential and de-inactivated by membrane hyperpolarization. The properties of this Ca^{2+} conductance were similar to those reported for inferior olive (Llinás & Yarom, 1981), thalamic (Jahnsen & Llinás, 1984) and spinal dorsal horn (Murase & Randic, 1983) neurones.

The bursting response typical of medial septal neurones clearly distinguishes these cells from the other types recorded in the septum. In addition, this firing pattern has not been reported to occur in other areas of the mammalian CNS (Crill & Schwindt, 1983; Llinás, 1984). After blockade of Na^+ conductance with TTX, medial septal neurones could still generate a burst of Ca^{2+}-dependent spikes. This behaviour contrasts with that of dorsolateral septal neurones which in the same experimental conditions generated Ca^{2+} spikes repetitively. This electrical property of medial septal neurones is most probably a consequence of the activation of a fast transient Ca^{2+}-dependent K^+ current that limits their excitability and may be also responsible for the pronounced after-hyperpolarization present in the cells. A percentage of this conductance was inactivated at the resting potential and de-inactivated by negative current pulses. In addition, the membrane potential failed to return immediately to the resting level after a hyperpolarization. A transient K^+ current, referred to as A current, has been identified in a number of neurones (Neher, 1971; Connor & Stevens, 1971; Thompson, 1977; Gustafsson *et al.* 1982; Segal & Barker, 1984; Galvan & Sedlmeir, 1984) and is blocked by low concentrations of 4-AP. Thus, it is unlikely that the transient K^+ current of medial septal neurones is an A-current since it was unaffected by 4-AP and blocked by Co^{2+} and by removal of external Ca^{2+}. However, the existence of a Ca^{2+}-activated fast transient outward current in frog sympathetic neurones (MacDermott & Weight, 1982; Brown, Constanti & Adams, 1983) and some Ca^{2+}-dependence of the 4-AP-sensitive A-current of molluscan neurones have been reported (Junge, 1985). Recently, a 4-AP-insensitive and Ca^{2+}-dependent fast inactivating K^+ current has been found in guinea-pig hippocampal pyramidal neurones (Zbicz & Weight, 1985) and this may be similar to that in the medial septal neurones. In the medial septum the transient K^+ current was not strongly affected by TEA^+ (5 mM, not shown), but in the hippocampus it has been reported that it is depressed by a higher concentration (10 mM) of TEA^+ (Zbicz & Weight, 1985). Another possibility is that the onset of the conductance underlying the slow return to the baseline could be triggered by Ca^{2+} entry due to the de-inactivation of a fast Ca^{2+} conductance by a membrane hyperpolarization. The increase in cytosolic Ca^{2+} could activate a Ca^{2+}-dependent K^+ conductance, as in dorsolateral septal neurones. This would explain the insensitivity to 4-AP of the slow return to baseline, and the disappearance of this response in presence of Co^{2+} or by removal of external Ca^{2+}

All cells recorded in the septum had in common a restricted excitability due to

their intrinsic membrane properties, and showed a failure to fire fast action potentials repetitively at high frequencies. This unusual characteristic of septal cells may account for the fact that most septal units recorded in intact animals fire either tonically at a low frequency or generate rhythmical bursts of spikes (Petsche, Gogolak & Van Zwieten, 1965; McLennan & Miller, 1974; Vinogradova *et al.* 1980; Lebrun & Poulain, 1982). The rhythmical activity of septal cells has been studied extensively in intact animals because it is believed to act as the pacemaker of the hippocampal theta rhythm (Apostol & Creutzfeldt, 1974; McLennan & Miller, 1974; Vinogradova *et al.* 1980; Lamour *et al.* 1984). *In vitro* two of the septal cell types, intermediate and medial septal neurones, could fire bursts of action potentials. Medial septal neurones also generated a bursting response followed by a period of low excitability very similar to that observed *in vivo*, with a duration compatible with the frequency of the theta rhythm (2–6 Hz). Thus, we propose that the rhythmical activity that appears in the medial septum in intact animals could be the result of the intrinsic electrical properties of septal neurones accentuated by their synaptic inputs.

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REFERENCES

ALVAREZ DE TOLEDO, G. & LÓPEZ-BARNEO, J. (1986). Electrophysiology of medial septal (MS) neurones *in vitro*. *Neuroscience Letters*, suppl. 26, S497.

ANDY, O. J. & STEPHAN, H. (1961). Septal nuclei of the Soricidal (insectivores): cytoarchytectonic study. *Journal of Comparative Neurology* **117**, 251–273.

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

BLANKENSHIP, J. E. & KUNO, M. (1968). Analysis of spontaneous subthreshold activity in spinal motoneurons of the cat. *Journal of Neurophysiology* **31**, 196–209.

BROWN, T. H., FRICKE, R. A. & PERKEL, D. H. (1981). Passive electrical constants in three classes of hippocampal neurones. *Journal of Neurophysiology* **46**, 812–827.

BROWN, D. A., CONSTANTI, A. & ADAMS, P. R. (1983). Ca-activated potassium current in vertebrate sympathetic neurones. *Cell Calcium* **4**, 407–420.

BRÜCKE, F., PETSCHE, H., PILLAT, B. & DEISENHAMMER, F. (1959). Die Beeinflussung des Hippocampus-arousal-Reaktion beim Kanichen durch elektrische Reizung im Septum. *Pflügers Archiv* **269**, 319–338.

CAJAL, S. RAMON Y. (1911). *Histologie du Systéme Nerveux de l'Homme et des Vertébrés*. Paris: Maloine.

CONNORS, B. W., GUTNICK, M. J. & PRINCE, D. A. (1982). Electrophysiological properties of neocortical neurones *in vitro*. *Journal of Neurophysiology* **48**, 1302–1320.

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

CRILL, W. E. & SCHWINDT, P. C. (1983). Active currents in mammalian central neurons. *Trends in Neurosciences* June, 236–240.

DINGLEDINE, R. (1984). *Brain Slices*. New York: Plenum Press.

DE FRANCE, J. F., YOSHIHARA, H. & CHRONISTER, R. B. (1976). Electrophysiological studies of the septal nuclei, I. The lateral septal region. *Experimental Neurology* **53**, 399–419.

DUTAR, P., LAMOUR, Y. & JOBERT, A. (1985). Activation of identified septo-hippocampal neurones by noxious peripheral stimulation. *Brain Research* **328**, 15–21.

GALVAN, M. & SEDLMEIR, C. (1984). Outward currents in voltage-clamped rat sympathetic neurones. *Journal of Physiology* **356**, 115–133.

GREEN, J. D. & ARDUINI, A. (1954). Hippocampal electrical activity in arousal. *Journal of Neurophysiology* **17**, 533–557.

GUSTAFSSON, B., GALVAN, M., GRAFE, P. & WIGSTRÖM, H. (1982). A transient outward current in a mammalian central neurone blocked by 4-aminopyridine. *Nature* **299**, 252–254.

HAGIWARA, S., FUKADA, J. & EATON, D. S. (1974). Membrane currents carried by Ca, Sr and Ba in barnacle muscle fiber during voltage clamp. *Journal of General Physiology* **63**, 564–578.

JAHNSEN, H. & LLINÁS, R. (1984). Electrophysiological properties of guinea-pig thalamic neurones: an *in vitro* study. *Journal of Physiology* **349**, 205–226.

JÖELS, M., TWERY, M. J., SHINNICK-GALLAGHER, P. & GALLAGHER, J. P. (1986). Multiple actions of serotonin on lateral septal neurons in rat brain. *European Journal of Pharmacology* **129**, 203–204.

JUNGE, D. (1985). Calcium dependence of A-currents in perfused *Aplysia* neurones. *Brain Research* **346**, 249–300.

KOHLHARDT, M., BAUER, B., KRAUSE, H. & FLECKENSTEIN, A. (1973). Selective inhibition of the transmembrane Ca conductivity of mammalian myocardial fibres by Ni, Co and Mn ions. *Pflügers Archiv* **338**, 309–322.

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

LEBRUN, C. J. & POULAIN, D. A. (1982). Electrical activity of septal neurones during suckling and the milk ejection reflex in the lactating rat. *Experimental Brain Research* **47**, 203–208.

LEBRUN, C. J. & POULAIN, D. A. (1983). The role of the septum in the control of milk ejection reflex in the rat: effects of lesions and electrical stimulation. *Journal of Physiology* **339**, 17–32.

LLINÁS, R. (1984). Comparative electrobiology of mammalian central neurones. In *Brain Slices*, ed. DINGLEDINE, R., pp. 83–94. New York: Plenum Press.

LLINÁS, R. & SUGIMORI, M. (1980). Electrophysiological properties of *in vitro* Purkinje cell somata in mammalian cerebellar slices. *Journal of Physiology* **305**, 171–195.

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

LÓPEZ-BARNEO, J. & ARMSTRONG, C. M. (1983). Depolarizing response of rat parathyroid cells to divalent cations. *Journal of General Physiology* **82**, 269–294.

LÓPEZ-BARNEO, J. & LLINÁS, R. (1983). Electrophysiological properties of superior colliculus neurones in *in vitro* guinea pig brain stem slices. *Neuroscience Letters*, suppl. 14, S226.

LÓPEZ-BARNEO, J., ALVAREZ DE TOLEDO, G. & YAROM, Y. (1985). Electrophysiological properties of guinea-pig septal neurons *in vitro*. *Brain Research* **347**, 358–362.

LUPARELLO, T. J. (1967). *Stereotaxic Atlas of the Forebrain of the Guinea Pig*. Baltimore: Williams & Wilkins.

MACDERMOTT, A. B. & WEIGHT, F. F. (1982). Action potential repolarization may involve a transient Ca^{2+}-sensitive outward current in a vertebrate neurone. *Nature* **300**, 185–188.

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

MCLENNAN, H. & MILLER, J. J. (1976). Frequency-related inhibitory mechanisms controlling rhythmical activity in the septal area. *Journal of Physiology* **254**, 827–841.

MEIBACH, R. C. & SIEGEL, A. (1977). Efferent connections of the septal area in the rat: an analysis utilizing retrograde and anterograde transport methods. *Brain Research* **119**, 1–20.

MURASE, K. & RANDIC, M. (1983). Electrophysiological properties of rat spinal dorsal horn neurones *in vitro*: calcium-dependent action potentials. *Journal of Physiology* **334**, 141–153.

NEHER, E. (1971). Two fast transient current components during voltage clamp on snail neurons. *Journal of General Physiology* **58**, 36–53.

PETSCHE, H., GOGOLAK, G. & VAN ZWIETEN, P. A. (1965). Rhythmicity of septal cell discharges at various levels of reticular excitation. *Electroencephalographic and Clinical Neurophysiology* **19**, 25–33.

RAISMAN, G. (1966). The connections of the septum. *Brain* **89**, 317–348.

SEGAL, M. & BARKER, J. L. (1984). Rat hippocampal neurons in culture: potassium conductances. *Journal of Neurophysiology* **51**, 1409–1433.

SCHWARTZKROIN, P. A. & SLAWSKY, M. (1977). Probable calcium spikes in hippocampal neurones. *Brain Research* **135**, 157–161.

STEVENS, D. R., GALLAGHER, F. & SHINNICK-GALLAGHER, P. (1984). Intracellular recordings from rat dorsolateral septal neurones, *in vitro*. *Brain Research* **305**, 353–356.

THOMPSON, S. H. (1977). Three pharmacologically distinct potassium conductances in molluscan neurones. *Journal of Physiology* **265**, 465–488.

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

ZBICZ, K. L. & WEIGHT, F. F. (1985). Transient voltage and calcium-dependent outward currents in hippocampal CA3 pyramidal neurons. *Journal of Neurophysiology* **53**, 1038–1058.