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MEMBRANE PROPERTIES AND SYNAPTIC POTENTIALS OF THREE TYPES OF NEURONE IN RAT LATERAL AMYGDALA

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SUMMARY

1. Intracellular recordings were made from the lateral nucleus of the amygdala in tissue slices cut from rat brain and maintained *in vitro*.

2. Three types of neurones were distinguished according to the after-potential that followed an action potential. Type 1 cells (44%, $n = 225$) had depolarizing after-potentials, resulting from a calcium-dependent chloride conductance. Type 2 cells (48%) had long-lasting (> 250 ms) hyperpolarizing after-potentials and type 3 cells (8%) had shorter hyperpolarizing after-potentials. The average resting potentials of the three cell types were −78, −69 and −62 mV respectively. Intracellular labelling with biocytin showed that type 1 cells were pyramidal neurones; type 2 and type 3 cells were non-pyramidal.

3. Experiments with receptor antagonists identified synaptic potentials mediated by excitatory amino acids and by GABA (acting at $GABA_A$ receptors) in all three cell types. A longer duration inhibitory synaptic potential resulting from activation of $GABA_B$ receptors was present in type 1 (pyramidal) and type 2 cells.

4. Cholecystokinin (100 nM to 1 μM) depolarized type 2 and type 3 cells but had no effect on type 1 (pyramidal) cells. Baclofen (1–3 μM) hyperpolarized type 1 and type 2, but not type 3 cells. [Met^5]enkephalin (1–10 μM) hyperpolarized only type 2 cells.

5. It is concluded that the lateral nucleus of the amygdala contains pyramidal neurones and two types of non-pyramidal neurone; these can be differentiated by membrane properties, synaptic inputs and sensitivities to transmitters.

INTRODUCTION

The basolateral amygdaloid group is one of four main subdivisions of the amygdala, the others being the olfactory amygdala (including the cortical nuclei), the medial amygdaloid group and the central amygdaloid group (de Olmos, Alheid & Beltramino, 1985). In the rat, it comprises almost 50% of the cells, and the other subdivisions have traditionally been grouped together as corticomedial groups (Gurdjian, 1928; Brodal, 1947). It receives input from the thalamus, provides

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significant outputs to the dorsal and ventral striatum, and has strong connections with cortical areas: this has led to it being considered more as a 'cortical' structure than part of the 'limbic' system (reviewed by Carlsen, 1989), a view for which there is also embryological evidence (Bayer, 1980). The basolateral amygdaloid group itself is generally divided into four main nuclei: lateral, basolateral, ventral basolateral and basomedial.

There have been several studies of the histology and cytoarchitectonics of the neurones of the basolateral amygdala (McDonald, 1982; Millhouse & de Olmos, 1983; reviewed by de Olmos *et al.* 1985). These reports indicate three main types of intrinsic neurone, closely resembling the cells of the cerebral cortex. In contrast, there have been no extensive investigations of the physiological properties of these neurones using the methods of intracellular recording. The few available reports have focused on the increased excitability seen when bicuculline or picrotoxin is used to block inhibitory synapses (Gean & Shinnick-Gallagher, 1987, 1989), a subject of considerable importance given that the basolateral amygdala seems to be particularly susceptible to epileptic discharge (Ben-Ari, Tremblay & Ottersen, 1980; Racine & Burnham, 1984). Thus, the general purpose of the present work was to determine whether different classes of neurone could be distinguished in the basolateral amygdaloid group on the basis of their membrane properties, synaptic potentials and sensitivity to transmitters, and to correlate this knowledge with histological identification by intracellular staining.

METHODS

Tissue preparation. Adult rats were anaesthetized with halothane and killed by a heavy blow to the chest. The brain was rapidly removed and a block of tissue containing the amygdaloid complex was sectioned with a vibratome. Horizontal slices were cut (300 μm) and the lateral amygdala was identified as a triangular wedge of grey matter between the lateral ventricle and the dorsal endopiriform nucleus. In some experiments, coronal slices were used (350 μm); in this case the lateral amygdala was recognized between the corpus callosum and the caudate/putamen. The extracellular solution contained (mM): NaCl 126, NaH_2PO_4 1·2, $MgCl_2$ 1·2, $CaCl_2$ 2·4, KCl 2·5, $NaHCO_3$ 25, glucose 11; gassed with 95% O_2 and 5% CO_2 (temperature 36 °C).

Electrical recordings,. Intracellular recordings were made using glass microelectrodes filled with 2 M KCl or 2 M potassium acetate with resistance 80–180 MΩ. Potentials were amplified (Axoclamp 2) and recorded on a chart recorder (Gould 2400). Presynaptic fibres were stimulated electrically with a bipolar tungsten-in-glass electrode, the tip of which was placed on the surface of the slice.

Histology. In some experiments, the recording electrode was filled with a solution containing KCl (2 M), Tris (50 mM, pH 7·2) and biocytin (1·5–2%). In these cases, the impaled neurone was allowed to fill for at least 30 min, and only one neurone was labelled in each slice. The slice was then fixed overnight (Zamboni fixative), washed and cut into 60 μm sections. These sections were incubated with rhodamine- or peroxidase-conjugated streptavidin (Jackson ImmunoResearch Laboratories) overnight and washed with phosphate buffer. The slices were mounted in buffered glycerol and examined using a fluorescence microscope (rhodamine) or reacted with diaminobenzidine and examined by transmitted light (peroxidase).

Solutions and drugs. Changes in potassium and calcium concentrations were made with equimolar substitution of sodium and magnesium respectively. Changes in sodium concentration were made by Tris substitution and changes in chloride concentration were made with sodium isethionate. Drugs were 2-hydroxysaclofen, 6-cyano-2, 3-dihydroxy-7-nitro-quinoxaline (CNQX; from Research Biochemicals Incorporated and Cambridge Research Biochemicals), DL-2-aminophosphonovaleric acid (APV), L-aspartic acid β-hydroxyamate, bicuculline, picrotoxin, baclofen, 5-hydroxytryptamine (5-HT), pentagastrin, cholecystokinin (CCK), sulphated cholecystokinin (CCK8S), [Met^5]enkephalin, Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO), Tyr-D-Pen-

Gly-Phe-D-Pen (DPDPE) (from Peninsula or Sigma), *trans*-(±)-3,4-dichloro-*N*-methyl-[2-(1-pyrrolidinyl)cyclohexyl] benzeneacetamide methane sulphonate (U50488H, Upjohn). ω-conotoxin (Bachem), and GR67330 (gift from Glaxo).

RESULTS

It became clear during the course of recordings that neurones could be distinguished according to several properties. The most obvious differentiating feature was the after-potential that followed an action potential (Fig. 1*A–C*); this was depolarizing in about one-half of the cells (termed type 1) and hyperpolarizing in others (Table 1). The cells that had hyperpolarizing after-potentials could be further differentiated into those in which this exceeded 250 ms duration (type 2) and those in which it did not (type 3). The after-potential therefore served as a convenient way to classify the cells but, as will be presented below, there were also differences in other membrane properties as well as in the response to agonists.

Passive membrane properties

Resting potentials were estimated by sudden withdrawal of the recording electrode; their distribution was biphasic with peaks at −78·3 and −63·8 mV (Fig. 1*D*). Input resistances were measured from the amplitude of hyperpolarizing electrotonic potentials (< 20 mV) and time constants were estimated from the time required for those electrotonic potentials to attain 63% of their final steady-state value.

Type 1 cells, defined by the presence of a depolarizing after-potential, had a resting potential of $-78{\cdot}2 \pm 0{\cdot}6$ mV ($n = 100$; 44% of total). This value coincides with the mean resting potential of the more polarized group of neurones obvious from the distribution of all the resting membrane potentials (Fig. 1). The input resistance of a sample of these cells was $80{\cdot}2 \pm 4{\cdot}9$ MΩ ($n = 35$) and time constant $36{\cdot}2 \pm 2{\cdot}8$ ms ($n = 33$). Type 2 cells had a resting membrane potential of $-68{\cdot}8 \pm 0{\cdot}7$ mV ($n = 107$; 48% of total), an input resistance of $93{\cdot}3 \pm 6{\cdot}1$ MΩ ($n = 40$) and a time constant of $43{\cdot}5 \pm 5{\cdot}2$ ms ($n = 35$). The membrane potential is significantly lower than that of type 1 cell ($P < 0{\cdot}001$, unpaired *t* test) but the resistances and time constants are not different. Type 3 neurones had a membrane potential of $-62{\cdot}1 \pm 1{\cdot}3$ mV ($n = 18$, 18% of total), input resistance of $91{\cdot}3 \pm 5{\cdot}8$ MΩ ($n = 8$) and time constant of $40{\cdot}2 \pm 4{\cdot}6$ ms ($n = 7$). The resting potential is less than that of type 2 cells ($P < 0{\cdot}01$, unpaired *t* test) but the other values are not significantly different. The differences are summarized in Table 1.

Action potentials and their after-potentials

The action potential of type 1 neurones had a mean amplitude of $68{\cdot}2 \pm 2{\cdot}1$ mV (measured from threshold) and a mean duration of $1{\cdot}2 \pm 0{\cdot}1$ ms ($n = 44$). The action potential was followed by an after-depolarization (30–150 ms) and an after-hyperpolarization (80–300 ms). The amplitude of the after-depolarization was increased by membrane hyperpolarization, and decreased by depolarization. Depolarization to $-53{\cdot}5 \pm 1{\cdot}3$ mV ($n = 6$) annulled the after-depolarization, but further depolarization failed to reverse its polarity (Fig. 2*A*). This potential is close to the reversal potential for the bicuculline-sensitive postsynaptic potential (see

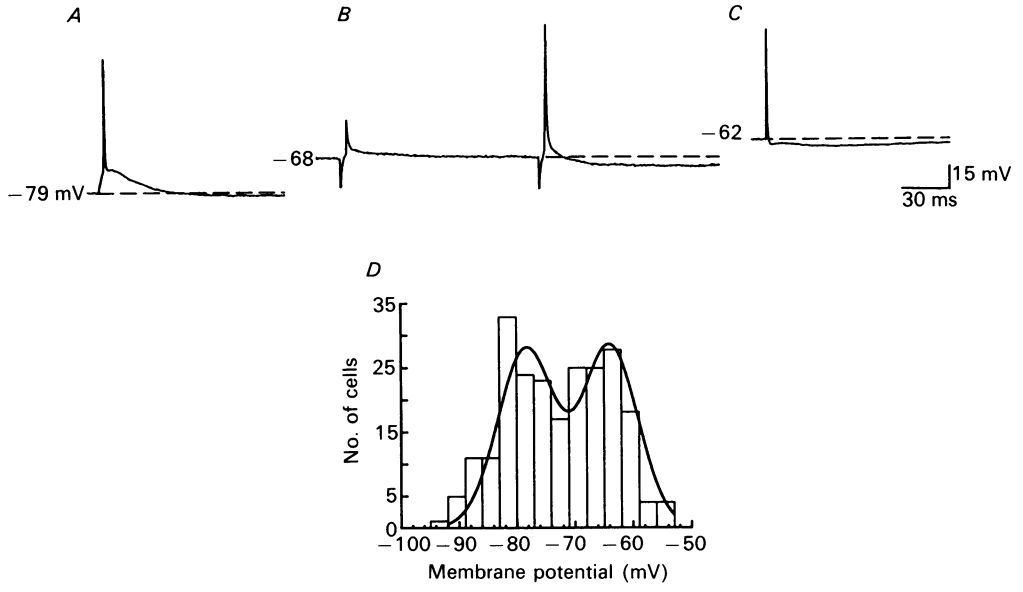

Fig. 1. *A–C*, after-potentials distinguish three types of neurone. The three traces illustrate the after-depolarization, long and short after-hyperpolarization used to distinguish type 1, type 2 and type 3 neurones. Action potentials were evoked by passing a brief depolarizing current through the recording electrode. Resting potentials were −79, −68 and −62 mV. In *B* the first depolarizing current pulse failed to evoke an action potential, and there was no after-potential. *D*, distribution of resting potentials. Potentials were binned in 3 mV increments; continuous line shows the fit to the sum of two Gaussians centred at −78·2 and −63·8 mV.

TABLE 1. Summary of properties of lateral amygdala neurones

	Type 1	Type 2	Type 3
Histology	Pyramidal	Non-pyramidal	Non-pyramidal
Membrane properties			
Resting potential (mV)	−78	−69	−62
Action potential duration (ms)	1·4	1·2	0·9
After-potential	Depolarizing	Hyperpolarizing	Hyperpolarizing
Synaptic inputs			
Excitatory amino acid	+	+	+
GABA at $GABA_A$ receptor	+	+	+
5-HT at $5\text{-}HT_3$ receptor*	+	−	−
GABA at $GABA_B$ receptor*	+	+	−
Agonist actions			
CCK/pentagastrin	No effect	Depolarizes	Depolarizes
Baclofen	Hyperpolarizes	Hyperpolarizes	No effect
Opioid	No effect	Hyperpolarizes	No effect

* Stimulation in region of basolateral nucleus.

below; $-47{\cdot}6 \pm 2{\cdot}2$ mv, $n = 4$). The after-depolarization was not affected by changing the extracellular potassium concentration (8·5 or 0·25 mM, $n = 8$), nor by reducing the extracellular sodium concentration to 38 mM ($n = 3$).

The amplitude of the after-depolarization was increased (to 128 ± 6% of control,

$n = 7$) after 5 min superfusion with a solution that contained a low (30 mM) chloride concentration; after 10 min of superfusion the after-depolarization was decreased to $60 \pm 8\%$ ($n = 4$) of control. The depolarization evoked by a brief superfusion with GABA (10 s, 1 mM) behaved similarly; it was increased to $139 \pm 10\%$ of control after

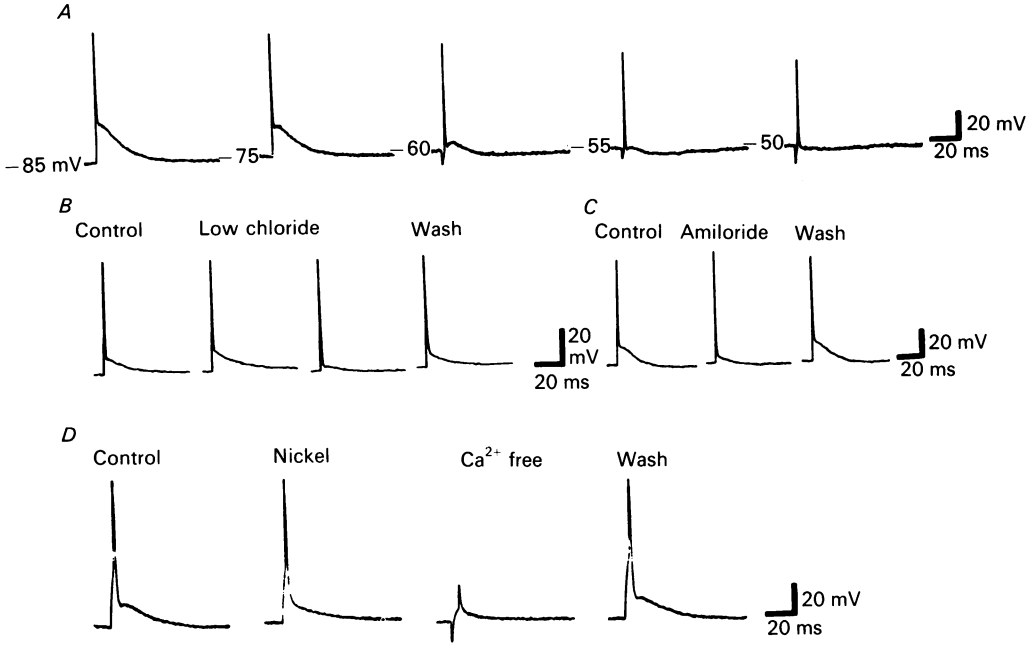

Fig. 2. After-depolarization of type 1 cells. *A*, the amplitude of the after-depolarization was increased by hyperpolarization and decreased by depolarization. Depolarization to −50 mV nullified the after-depolarization. Resting potential −75 mV. *B*, after-depolarization was first increased and then decreased in solutions containing low chloride concentration. The second and third traces were recorded 5 and 10 min after changing to a solution that contained 30 mM chloride. *C*, amiloride (10 μM) reversibly reduces the after-depolarization. Resting potential was −72 mV. *D*, action potentials in tetrodotoxin (1 μM) are followed by after-depolarization. Nickel (100 μM) did no affect the action potential but reduced the after-depolarization. Calcium-free solution abolished action potential and the after-depolarization. Resting potential was −77 mV.

5 min in the low chloride concentration, but decreased to $59 \pm 9\%$ of control after 10 min. These changes were reversible.

Calcium-free solution ($n = 10$) reversibly blocked the after-depolarization, as did amiloride (10 μM, $n = 3$) and nickel (10 μM, $n = 6$), which are somewhat selective for low-voltage-activated calcium conductances (Tsien, Lipscombe, Madison, Bley & Fox, 1988). Nifedipine (3 μM, $n = 5$), gadolinium (10–30 μM, $n = 3$) and ω-conotoxin (1 μM, $n = 3$) had no effect. The effect of calcium-free solutions raised the possibility that the after-depolarization might result from, or have a contribution from, a recurrent synaptic potential; however, CNQX (10 μM), APV (30 μM), bicuculline (30 μM) and the 5-HT receptor blocker GR 67330 (1 μM), which blocked evoked synaptic potentials (see Sugita, Shen & North, 1992*a*), did not affect the after-depolarization.

Tetrodotoxin (1 μM) blocked the action potential, but this could be restored by an increase in the size of the depolarizing current pulse. This restored action potential was also followed by an after-depolarization; both the action potential and the after-depolarization were reversibly blocked by calcium-free solution or by cobalt (2 mM).

Type 2 neurones ($n = 33$) had action potentials $1{\cdot}4 \pm 0{\cdot}1$ ms in duration and $72{\cdot}3 \pm 3{\cdot}5$ mV in amplitude; the action potentials were followed by a long-lasting (300–800 ms) after-hyperpolarization. Type 3 cells ($n = 15$) had action potentials of shorter duration ($0{\cdot}9 \pm 0{\cdot}2$ ms) and smaller amplitude ($55{\cdot}2 \pm 4{\cdot}7$ mV) that were followed only by a short after-hyperpolarization (30–150 ms). The action potential of these cells was significantly shorter than those of type 1 or type 2 cells (Table 1).

Actions of cholecystokinin and pentagastrin

Cholecystokinin (CCK) immunoreactivity is present in cells of the basolateral amygdala (Roberts, Woodhams, Polak & Crow, 1982) where it co-exists with GABA in a class of non-pyramidal cell (McDonald & Pearson, 1989). We therefore determined whether the different classes of cell were selectively sensitive to the CCK analogues 8-sulphated CCK (CCK8S, an agonist at both CCK-A and CCK-B receptors) and pentagastrin (a more selective CCK-B agonist).

Eight of ten type 1 cells showed no effect of CCK8S or pentagastrin (300 nM–1 μM). Two neurones were hyperpolarized (2–4 mV) with a reduction of input resistance; this hyperpolarization was diminished by cadmium (300 μM) or 2-hydroxysaclofen (300 μM) and was therefore assumed to result from the release of GABA from other cells in the slice that were excited by CCK.

Sixteen of twenty-one type 2 cells were depolarized by both agonists (100 nM–1 μM) (Fig. 3*A*). The depolarization was small ($2{\cdot}2 \pm 0{\cdot}3$ mV) but reproducible, and was associated with an increase in input resistance. The response desensitized during applications persisting for more than 3 min. This depolarization was unaffected by cadmium (300 μM, $n = 3$) and TTX (1 μM, $n = 2$). The depolarization evoked by CCK became larger when the membrane was depolarized by current injection during the CCK application; together with the increase in input resistance, this suggests a decrease in potassium conductance. Such an effect of CCK has previously been described in neurones of the ventromedial hypothalamus (Boden & Hill, 1988).

Type 3 neurones were also depolarized ($4{\cdot}2 \pm 1{\cdot}6$ mV, three of four cells) by CCK8S and pentagastrin (100 nM–1 μM), together with an increase in input resistance (Fig. 3*A*). The action of the agonists appeared to reverse more slowly on washing (5–7 min) than was seen for type 2 cells (2–3 min) (Fig. 3*A*).

Actions of opioids and baclofen

The three types of neurone in the basolateral amygdala could also be distinguished by their responses to opioid peptides and baclofen (Fig. 3*B*; Table 1).

Baclofen (1–3 μM) hyperpolarized type 1 neurones by $6{\cdot}4 \pm 2{\cdot}1$ mV ($n = 7$) whereas [Met^5]enkephalin (30 μM) had no effect ($n = 10$). The action of baclofen was associated with a decrease in input resistance. Agonists selective for different types of opioid receptor were also ineffective DAMGO (1–3 μM, $n = 5$), DPDPE (1–3 μM, $n = 5$) and U50448H (30 μM, $n = 5$).

All of ten type 2 neurones tested were hyperpolarized ($9{\cdot}8 \pm 1{\cdot}0$ mV) by baclofen

(3 μM), and seven of nine cells were also hyperpolarized by [Met^5]enkephalin (3–100 μM). DAMGO (1–3 μM) also hyperpolarized these neurones (19 of 25 tested); at 3 μM the hyperpolarization was 9·2 ± 0·6 mV ($n = 8$). DPDPE (1–3 μM, $n = 7$) and U50448H (30 μM, $n = 3$) had no effect. Hyperpolarizations by opioids and by baclofen were associated with a decrease in input resistance.

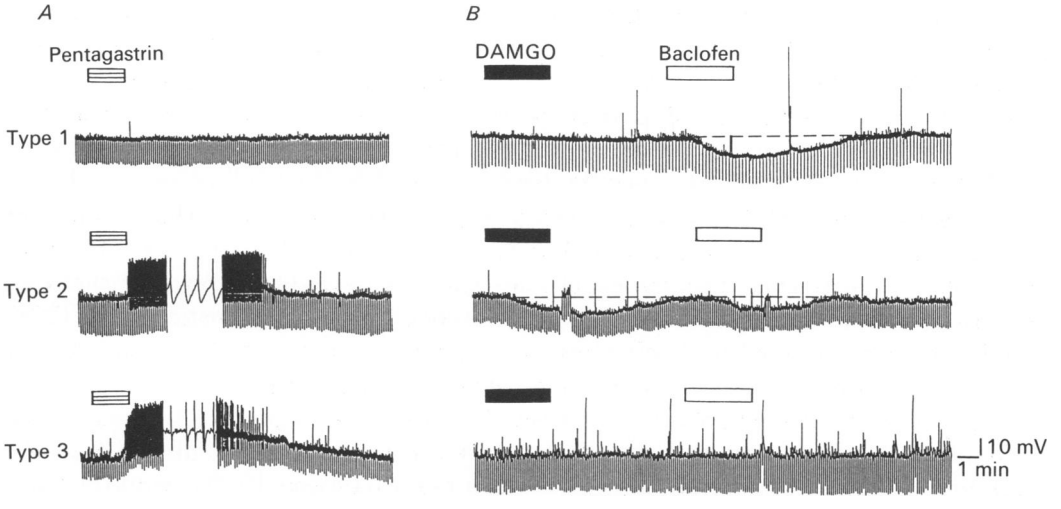

Fig. 3. *A*, pentagastrin (1 μM), an agonist at CCK-B receptors, had no effect on a type 1 cell (top record) but depolarized type 2 and type 3 neurones (middle and bottom records). Resting potentials were −76, −62 and −65 mV, respectively. *B*, type 1 neurone (upper record) was hyperpolarized by the $GABA_B$ agonist baclofen (open bars) but not affected by the opioid agonist DAMGO (filled bars); resting potential was −73 mV. Type 2 neurone was hyperpolarized by both DAMGO and baclofen (middle record; resting potential was −65 mV). Type 3 neurone was unaffected by either DAMGO or baclofen (bottom record, resting potential −60 mV).

Neither baclofen (3 μM, $n = 3$) nor opioids ([Met^5]enkephalin, 30 μM, $n = 2$; DAMGO, 3 μM, $n = 3$) affected the membrane potential of type 3 cells.

Synaptic potentials

The responses to electrical stimulation of the tissue slice depended both on the type of neurone, and on the position of the tip of the stimulating electrode.

In seventy-eight type 1 cells tested, a synaptic potential was elicited by a single focal stimulus (0·1–0·3 ms) applied to the surface of the *lateral* nucleus of the amygdala. The amplitude of the synaptic potential was graded with the stimulus intensity. This synaptic potential was blocked by tetrodotoxin (1 μM), cobalt (2 mM), cadmium (500 μM) or calcium-free solution. When studied at the resting membrane potential, bicuculline (30 μM) or picrotoxin (100 μM) typically reduced the amplitude of the synaptic potential by about 30%; the effect of bicuculline was reversible but that of picrotoxin was not. A combination of blockers of excitatory amino acid receptors (10 μM CNQX and 30 μM APV) reduced the synaptic potential by 70%.

The excitatory amino acid component was isolated with bicuculline (30 μM) or picrotoxin (100 μM); it had a time to peak of 4·8 ± 1·6 ms and a duration of

$104 \pm 8{\cdot}3$ ms ($n = 20$). The amplitude and the duration of these synaptic potentials were increased by L-aspartate-β-hydroxamate (50 μM, $n = 4$), which inhibits the uptake of excitatory amino acids; the synaptic potential was abolished by a combination of CNQX (10 μM) and APV (30–100 μM) (Fig. 4*A*). The synaptic potential was quite reproducible in most cells when the stimulus was repeated at low frequency (e.g. 1 Hz). However in 20% neurones a progressive increase in amplitude of the synaptic potential was observed, until action potentials were evoked by a stimulus that was originally subthreshold. This phenomenon was prevented by APV (30 μM) or by muscarine (30 μM).

A component resulting from $GABA_A$ receptor activation was isolated with CNQX (10 μM) and APV (30 μM); it had a time-to-peak of $6{\cdot}7 \pm 1{\cdot}2$ ms and a duration of $52{\cdot}4 \pm 3{\cdot}5$ ms ($n = 20$). It was completely blocked by bicuculline or picrotoxin. This synaptic potential reversed polarity at $-47{\cdot}6 \pm 2{\cdot}2$ ($n = 4$) when electrodes contained potassium chloride and at $-72{\cdot}6 \pm 1{\cdot}5$ ($n = 3$) with electrodes containing potassium acetate (Fig. 4*B*). Spontaneously occurring synaptic potentials that had a time course similar to the evoked potential were observed in almost all cells. These were reversibly blocked by bicuculline.

Similar synaptic potentials were also evoked when the stimulating electrode was positioned over the *basolateral* nucleus of the amygdala. However, two additional synaptic potentials were now sometimes observed, in the presence of CNQX, APV and bicuculline or picrotoxin. The first was a depolarization 15–70 ms in duration that was blocked by antagonists at 5-HT_3 receptors (see Sugita *et al.* 1992*a*). The second was a slow hyperpolarizing potential with a duration of 350–1500 ms. This synaptic potential was mediated by $GABA_B$ receptors because it was enhanced by nipecotic acid (1 mM) and blocked by 2-hydroxysaclofen (100–300 μM) (Fig. 4*C*). This slow synaptic potential resulted from an increase in potassium conductance because its polarity reversed at $-108{\cdot}2 \pm 2{\cdot}4$ mV ($n = 6$) in 2·5 mM potassium (Fig. 4*D*). The reversal potential was linearly related to the logarithm of the extracellular potassium concentration (55 mV per decade, 2·5–10·5 mM potassium).

Single focal stimulation applied to the surface of the *lateral* amygdala evoked synaptic potentials in type 2 neurones similar to those described above, with components mediated by excitatory amino acid and by $GABA_A$ receptors; however, there was no residual fast synaptic potential sensitive to 5-HT_3 receptor blockers. A brief train of stimuli applied to the *basolateral* nucleus evoked also a slow hyperpolarizing synaptic potential mediated by the $GABA_B$ receptor. In about 30% of these cells, spontaneously occurring synaptic potentials were observed that were depolarizing and blocked by bicuculline or hyperpolarizing and blocked by 2-hydroxysaclofen (see Sugita, Johnson & North, 1992*b*). The hyperpolarizing synaptic potentials were seen only slices cut in the coronal plane, and not in slices cut horizontally; they were blocked by TTX (1 μM).

Type 3 neurones appeared to receive only excitatory amino acid and $GABA_A$ type synaptic inputs. Even trains of stimuli applied in the *basolateral* amygdala did not evoke any 5-HT_3 or $GABA_B$ component.

Synaptic potentials in all types of cell were blocked by changing to a calcium-free solution or by agents expected to prevent calcium entry into presynaptic nerve terminals. Indirect information about the class of channel present on these terminals was sought be examining the actions of

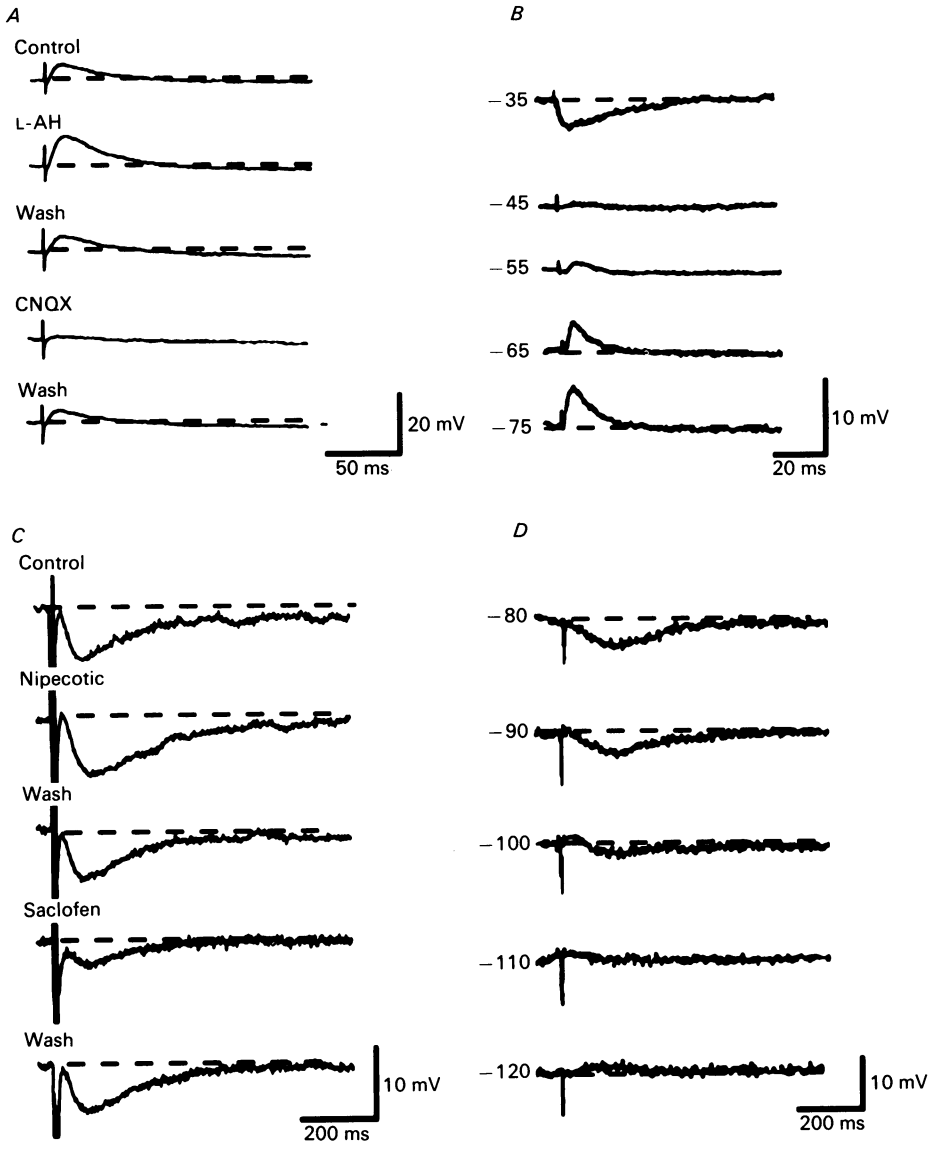

Fig. 4. Glutamate-, $GABA_A$- and $GABA_B$-mediated synaptic potentials. *A*, recordings from a type 1 cell, in bicuculline (30 μM). The EPSP was enhanced by L-aspartate-β-hydroxamate (L-AH; 50 μM) and blocked by CNQX (10 μM) and APV (30 μM). Resting potential was −79 mV. *B*, recording from a type 2 neurone, in CNQX (10 μM) and APV (30 μM). The EPSP reversed polarity at −48 mV (electrode contained potassium chloride). Potential (mV) beside each trace; resting potential was −65 mV. *C*, slow IPSPs were increased by nipecotic acid (1 mM) and blocked by 2-hydroxysaclofen (300 μM). Type 2 neurone. Potential (mV) beside each trace; resting potential was −65 mV. *D*, reversal of slow IPSP at about −110 mV in normal extracellular potassium. Type 1 neurone; resting potential was −80 mV.

several such blocking agents with some selectivity for different classes of calcium channel (Tsien *et al.* 1988). The component of the synaptic potential mediated by excitatory amino acids (termed the glutamate component) was isolated by bicuculline (30 μM) or picrotoxin (100 μM); in other experiments the component that was mediated by GABA was isolated by CNQX (10 μM) and APV

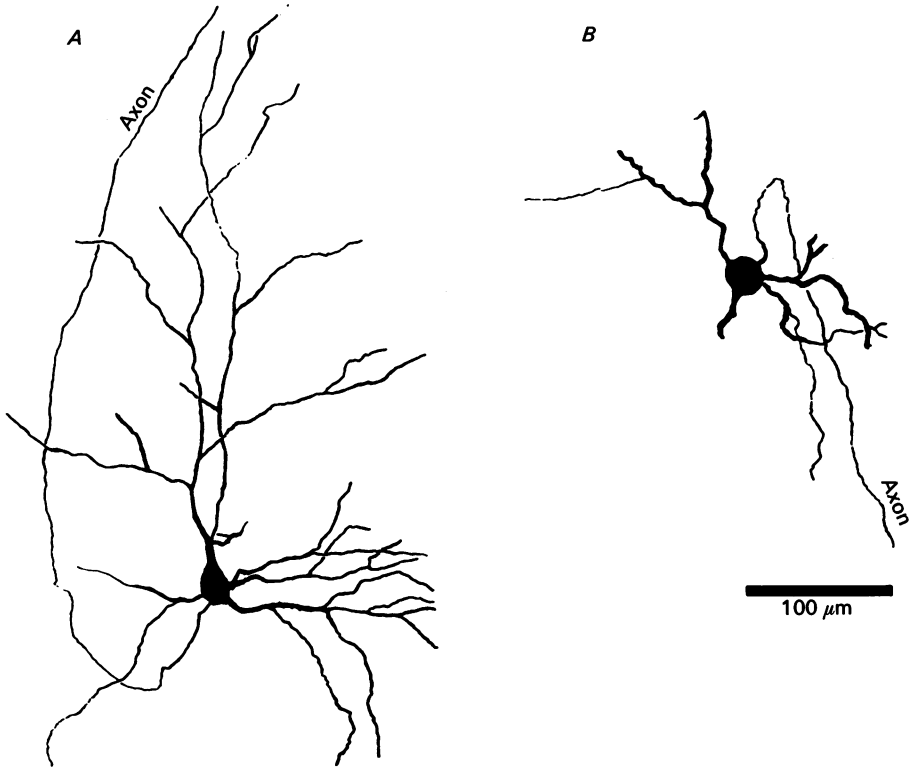

Fig. 5. Intracellular staining. Camera lucida drawings of two lateral amygdala neurones. *A*, a pyramidal cell. This cell showed an after-depolarization. *B*, a non-pyramidal cell. This neurone had an after-hyperpolarization following the action potential.

(30 μM). Gadolinium and nickel were about equally effective at inhibiting both glutamate- and GABA-mediated synaptic potentials; the concentrations that reduced the synaptic potential amplitude by 50% (EC_{50}) were about 8 μM and 8 mM respectively. Cadmium was more effective at reducing the glutamate (EC_{50} 30 μM) than the GABA component (EC_{50} 100 μM). ω-Conotoxin showed the reverse selectivity; at 1 μM it reduced the glutamate component by $37{\cdot}8 \pm 8 \pm 4{\cdot}1\%$ ($n = 5$) and the GABA component by $71{\cdot}3 \pm 4{\cdot}4\%$ ($n = 4$); the action was not reversible after 1 h washing. Nifedipine (1 μM, $n = 5$) and Bay K 8644 (1 μM, $n = 5$) had no effect on the synaptic potentials.

Intracellular staining

Twenty cells were filled with biocytin and labelled with rhodamine- or peroxidase-conjugated streptavidin. Type 1 cells had a pyramidal cell body (about 22×10 μm) with an apical dendrite five or six primary dendrites (Fig. 5*A*). These neurones were similar in appearance to those termed class 1 by McDonald (1982). Type 2 cells were round or oval in shape and had four to seven dendrites (Fig. 5*B*). The cell body was

approximately 16 × 20 μm. Several secondary dendrites arose from primary dendrites and could be followed for up to 500 μm. Type 3 cells also had non-pyramidal, oval shapes ($n = 2$) with cell bodies about 14 × 10 μm. Five or six primary dendrites were observed.

DISCUSSION

The lateral nucleus of the amygdala is one of the four divisions of the basolateral amygdaloid complex (see Introduction). Previous histological studies have described two major cell types, pyramidal and non-pyramidal (McDonald, 1982; de Olmos *et al.* 1985 and references therein). Pyramidal neurones had cell bodies about 20 μm in longest axis and strikingly resemble pyramidal cells of the cerebral cortex (McDonald, 1982). Non-pyramidal neurones can be further divided into two main groups: one has round, smooth cell bodies (approximately 15 μm) and is immunoreactive for the GABA-synthetic enzyme glutamic acid decarboxylase whereas the other has smooth, fusiform cell bodies (approximately 20 μm) and is peptide-immunoreactive (McDonald, 1982, 1985*a*, *b*, 1989). The present results, in which biocytin was injected into a sample of cells, indicate that type 1 cells are pyramidal neurones and that type 2 and type 3 cells are not; insufficient cells were stained to make further distinction between type 2 and type 3 neurones.

Pyramidal neurones

The most useful distinguishing feature of the pyramidal cells was the after-depolarization that followed an action potential. Several lines of evidence indicate that this results from an inward calcium current that activates a chloride conductance. First, it became larger with hyperpolarization, smaller with depolarization and was nullified at about −50 mV, which is close to the reversal potential for GABA. Second, it became larger, at least initially, when the extracellular chloride ion concentration was reduced; so did the response to applied GABA. Superfusion for longer periods (10 min) with low chloride solutions reversibly inhibited both the after-depolarization and the response to GABA, perhaps because intracellular chloride concentration falls as a result of inhibition of an inwardly directed pump (e.g. Alvarez-Leefmans, Gamino, Giraldez & Nogueron, 1988). Third, it was reversibly blocked by removal of extracellular calcium, or by nickel or amiloride.

Pyramidal cells could also be distinguished in other ways from the other neurones of the lateral amygdala (Table 1). They were more polarized (Fig. 1), they were the only cells not depolarized by cholecystokinin and they were the only class of cell in which 5-HT_3 receptor-mediated synaptic potentials were observed (Sugita *et al.* 1992*a*). The saclofen-sensitive hyperpolarization induced by CCK in two cells is consistent with the view that GABA-containing interneurones are excited by CCK; these may be type 2 and/or type 3 cells. However, synaptic potentials evoked by GABA acting at $GABA_B$ receptors were most readily observed when the stimulating electrode was positioned over the basolateral nucleus, and Sugita *et al.* (1992*b*) found that local application of glutamate over the basolateral nucleus but not the lateral nucleus caused spontaneous $GABA_B$-mediated synaptic potentials in some pyramidal cells; the probable explanation is that some GABA-releasing neurones in the basolateral nucleus are also excited by CCK, and that these project onto pyramidal

cells in the lateral nucleus. The presence of $GABA_B$-mediated synaptic potentials correlates with the finding that pyramidal cells were hyperpolarized by baclofen.

The lack of effects of opioids on the pyramidal cells is reminiscent of the findings in the CA1 region of the hippocampus, in the olfactory bulb (Nicoll, Alger & Jahr, 1980) and the ventral tegmental area (Johnson & North, 1992). In these structures, local circuit interneurones are hyperpolarized by opiates and the output cells (pyramidal cells in the case of hippocampus) are excited through disinhibition. The excitation of amygdala neurones by systemic morphine *in vivo* (Chou & Wang, 1977) is consistent with this, if it is assumed that the cells from which their recordings were made were mostly the larger pyramidal neurones.

The other synaptic potentials recorded in pyramidal cells of the lateral amygdala (mediated at $GABA_A$ and excitatory amino acid receptors) did not serve to distinguish these cells from the non-pyramidal cells. With respect to their reversal potential, sensitivity to antagonists and uptake blockers, they were similar to the synaptic potentials recorded from neurones in most parts of the brain, including the central nucleus of the amygdala (Nose, Higashi, Inokuchi & Nishi, 1991). The amygdala is not known to receive inputs from long GABA-containing fibres (Le Gal La Salle, Paxinos, Emson & Ben Ari, 1978); thus it may be assumed that the GABA component results from excitation of local interneurones and the excitatory amino acid component results from excitation of terminals of incoming fibres from cortex and other regions (de Olmos *et al.* 1985).

Non-pyramidal neurones

The type 2 and type 3 cells are presumed to represent two of the several classes of interneurones that have been described within the lateral amygdala. The main differentiating features here were the expression of opioid and $GABA_B$ receptors, in the latter case evidenced both by responses to baclofen and the saclofen-sensitive synaptic potentials (Table 1). There was also a clear difference in the membrane properties; the broader action potential of the type 2 cell was followed by a longer lasting after-hyperpolarization (Table 1). Histologically, several different types of non-pyramidal cells have been described in the lateral amygdala (de Olmos *et al.* 1985). Millhouse & de Olmos (1983) reported that stellate cells were the most abundant non-pyramidal cells seen in Golgi-stained sections, and cone cells were the third main group. However, there is insufficient evidence to conclude that the present designation of the cells corresponds to these two groups, or to the class II and class III described in Golgi-impregnated material by McDonald (1982).

Non-pyramidal cells differ from the pyramidal neurones of the basolateral amygdala by their content of GABA (McDonald, 1985*a*; Nitecka & Ben-Ari, 1987), and many of these also stain for vasoactive intestinal polypeptide and CCK (McDonald & Pearson, 1989). A distinct set of non-pyramidal cells is immunoreactive for somatostatin and neuropeptide Y (McDonald, 1989). The present finding that these cells, but not the pyramidal neurones, are directly affected by CCK suggests a role for the peptide in the intrinsic circuitry of the lateral amygdala.

Both types of non-pyramidal cell receive synaptic inputs at excitatory amino acid receptors and at $GABA_A$ receptors. However, only in the more abundant type 2 cells were $GABA_B$ synaptic potentials observed; this suggests that type 2 and type 3 cells may take part in distinct intrinsic circuits. The basolateral amygdala contains both

cell bodies and fibres that are immunoreactive for pro-enkephalin-derived peptides (Khachaturian, Lewis, Schafer & Watson, 1985); the sensitivity of type 2 cells, but not type 1 (pyramidal) or type 3, may also indicate that enkephalin plays a role in the local circuitry of the basolateral amygdaloid.

The different classes of cells demonstrated by intracellular recording in the present work, and the general resemblance of their properties to those of the neocortex and hippocampal CA1 region, is consistent with the increasing consensus from anatomical studies that the basolateral amygdaloid complex is organized along similar principles to the cortex. The different cell properties and sensitivities to transmitters will be helpful in the eventual assembly of a more complete picture of the intrinsic organization of the amygdala, a picture that is needed to understand its role in integrative behaviour.

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REFERENCES

ALVAREZ-LEEFMANS, F. J., GAMINO, S. M., GIRALDEZ, F. & NOGUERON, I. (1988). Intracellular chloride regulation in amphibian dorsal root ganglion neurones studied with ion-sensitive electrodes. *Journal of Physiology* **406**, 225–246.

BAYER, S. A. (1980). Quantitative ^{3}H-thymidine radiographic analyses of neurogenesis in the rat amygdala. *Journal of Comparative Neurology* **194**, 845–875.

BEN-ARI, Y., TREMBLAY, E. & OTTERSEN, O. P. (1980). Injections of kainic acid into the amygdaloid complex of the rat: an electrographic clinical and histological study in relation to the pathology of epilepsy. *Neuroscience* **5**, 515–528.

BODEN, P. & HILL, R. G. (1988). Effects of cholecystokinin and related peptides on neuronal activity in the ventromedial nucleus of the rat hypothalamus. *British Journal of Pharmacology* **94**, 246–252.

BRODAL, A. (1947). The amygdaloid nucleus in the rat. *Journal of Comparative Neurology* **87**, 1–16.

CARLSEN, J. (1989). New perspectives on the functional anatomical organization of the basolateral amygdala. *Acta Neurological Scandinavica* **79**, suppl. 1–27.

CHOU, D. T. & WANG, S. C. (1977). Unit activity of amygdala and hippocampal neuron. Effects of morphine and benzodiazepines. *Brain Research* **126**, 427–440.

DE OLMOS, J., ALHEID, G. F., BELTRAMINO, C. A (1985). Amygdala. In *The Rat Nervous System*, vol. 1, ed. PAXINOS, G., pp. 223–334. Academic Press, Melbourne.

GEAN, P.-W. & SHINNICK-GALLAGHER, P. (1987). Picrotoxin-induced epileptiform activity in amygdaloid neurons. *Neuroscience Letters* **73**, 149–154.

GEAN, P.-W. & SHINNICK-GALLAGHER, P. (1989). The transient potassium current, the A-current, is involved in spike frequency adaptation in rat amygdala neurons. *Brain Research* **480**, 160–169.

GURDJIAN, E. S. (1928). The corpus striatum of the rat. Studies on the brain of the rat. *Journal of Comparative Neurology* **45**, 249–281.

JOHNSON, S. W. & NORTH, R. A. (1992). Opioids excite dopamine neurons by hyperpolarization of local interneurones. *Journal of Neuroscience* **12**, 483–488.

KHACHATURIAN, H., LEWIS, M. E., SCHAFER, M. K.-H. & WATSON, S. J. (1985). Anatomy of the CNS opioid systems. *Trends in Neurosciences* **8**, 111–119.

LE GAL LA SALLE, G., PAXINOS, G., EMSON, P. & BEN ARI, Y. (1978). Neurochemical mapping of GABAergic systems in the amygdaloid complex and bed nucleus of the stria terminalis. *Brain Research* **155**, 397–403.

MCDONALD, A. J. (1982). Neurons of the lateral and basolateral amygdaloid nuclei: A Golgi study in the rat. *Journal of Comparative Neurology* **212**, 293–312.

MCDONALD, A. J. (1985*a*). Immunohistochemical identification of γ-aminobutyric acid-containing neurons in the rat basolateral amygdala. *Neuroscience Letters* **53**, 203–207.

MCDONALD, A. J. (1985*b*). Morphology of peptide-containing neurons in the rat basolateral amygdaloid nucleus. *Brain Research* **338**, 186–191.

McDONALD, A. J. (1989). Coexistence of somatostatin with neuropeptide Y, but not with cholecystokinin of vasoactive intestinal peptide, in neurons of the rat amygdala. *Brain Research* **500**, 37–45.

McDONALD, A. J. & PEARSON, J. C. (1989). Coexistence of GABA and peptide immunoreactivity in non-pyramidal neurons of the basolateral amygdala. *Neuroscience Letters* **100**, 53–58.

MILLHOUSE, O. E. & DE OLMOS, J. (1983). Neuronal configurations in lateral and basolateral amygdala. *Neuroscience* **10**, 1269–1300.

NICOLL, R. A., ALGER, B. E. & JAHR, C. E. (1980). Enkephalin blocks inhibitory pathways in the vertebrate CNS. *Nature* **287**, 22–25.

NITECKA, L. & BEN-ARI, Y. (1987). Distribution of GABA-like immunoreactivity in the rat amygdaloid complex. *Journal of Comparative Neurology* **266**, 45–55.

NOSE, I., HIGASHI, H., INOKUCHI, H. & NISHI, S. (1991). Synaptic responses of guinea-pig and rat central amygdala neurons in vitro. *Journal of Neurophysiology* **65**, 1227–1241.

RACINE, R. J. & BURNHAM, W. M. (1984). The kindling model. In *Electrophysiology of Epilepsy*, ed. SCHWARTZKROIN, P. A. & WHEAL, A. V., pp. 153–171. Academic Press, London.

ROBERTS, G. W., WOODHAMS, P. L., POLAK, J. M. & CROW, T. J. (1982). Distribution of neuropeptides in the limbic system of the rat: the amygdaloid complex. *Neuroscience* **7**, 99–131.

SUGITA, S., SHEN, K.-Z. & NORTH, R. A. (1992*a*). 5-Hydrozytryptamine is a fast excitatory transmitter at 5-HT_3 receptors in rat amygdala. *Neuron* **8**, 199–203.

SUGITA, S., JOHNSON, S. W. & NORTH, R. A. (1992*b*). Synaptic inputs to $GABA_A$ and $GABA_B$ receptors originate from discrete afferent neurons. *Neuroscience Letters* **134**, 207–211.

TSIEN, R. W., LIPSCOMBE, D., MADISON, D. V., BLEY, K. R. & FOX, A. P. (1988). Multiple types of neuronal calcium channels and their selective modulation. *Trends in Neurosciences* **11**, 431–438.