DEVELOPMENTAL BIOLOGY 138, 313–323 (1990)

Changes in Messenger RNAs Coding for Neurotransmitter Receptors and Voltage-Operated Channels in the Developing Rat Cerebral Cortex

MELISSA K. CARPENTER,[1] IAN PARKER, AND RICARDO MILEDI

Laboratory of Cellular and Molecular Neurobiology, Department of Psychobiology, University of California, Irvine, California 92717

Accepted November 7, 1989

The ontogenetic development of poly(A)$^+$ mRNAs coding for receptors to several neurotransmitters (kainate, glutamate, acetylcholine, and serotonin) and voltage-operated channels (sodium and calcium) was studied by isolating total poly(A)$^+$ mRNA from the brains of rats at various developmental stages and injecting it into *Xenopus* oocytes. The oocytes translated the foreign mRNA and incorporated functional receptor/ion channel complexes into the cell membrane. Thus, recording of induced membrane currents in voltage-clamped oocytes gave a measure of the relative amounts of the different messengers. Responses induced by kainate, glutamate, acetylcholine, and serotonin all increased with age and reached a maximum in oocytes injected with mRNA from adult cortex. Messenger RNAs for the earliest ages examined, Embryonic Days 15 and 18, expressed little or no response to kainate, glutamate, or acetylcholine, while 50–70% of the adult response was reached by Postnatal Day 10. In contrast, the serotonin-induced response was already comparatively large (16% of the adult level) in oocytes injected with mRNA from Embryonic Day 15 brain and increased postnatally to adult levels. The expression of voltage-dependent sodium and calcium channels was small in oocytes injected with mRNA from embryonic animals and increased postnatally to reach a maximum in oocytes injected with mRNA from adult animals.

INTRODUCTION

The development of neurotransmitters and their receptors and of voltage-operated channels has been studied extensively in the brain (for reviews see Lanier *et al.*, 1976; Coyle, 1977, 1982; Telang and Enna, 1985; Hunt, 1988) and it is thought that different neurotransmitter receptors and membrane channels appear at different times during development (Johnson, 1985; Telang and Enna, 1985; Zilles *et al.*, 1985, 1986). Most of these studies used histochemical techniques, in particular, antibodies or radioactively labeled ligands. However, in many cases these techniques are restricted in their application because of a lack of specific probes, and interpretation is complicated because the ligands may bind to sites in addition to the physiological receptors or channels.

Instead of studying receptors and channels directly, we have used the *Xenopus* oocyte expression system to study the development of mRNAs which code for several receptors and channels. Briefly, this approach involves purifying poly(A)$^+$ mRNA from various ages of embryonic and postnatal rat cerebral cortex and injecting the mRNA into *Xenopus* oocytes. The oocytes will then translate the foreign mRNA and express many different functional receptors and voltage-operated channels (Gundersen *et al.*, 1983a,b; 1984a; Dascal, 1987; Miledi *et al.*, 1989). The membrane currents mediated by specific receptors and channels can then be recorded and used as an indicator of the amount of mRNA encoding a particular receptor or channel (Akagi and Miledi, 1988; Carpenter *et al.*, 1988a).

We have already used this approach to examine developmental changes in amounts of mRNAs coding for the inhibitory receptors to GABA and glycine in the rat brain (Carpenter *et al.*, 1988a), as well as various receptors and channels in chick optic lobe (I. Parker, K. Sumikawa and R. Miledi, unpublished). We now continue these studies and describe developmental changes in mRNAs encoding receptors activated by kainate, glutamate, serotonin (5-HT), and acetylcholine (ACh), as well as in mRNAs encoding voltage-operated sodium and calcium channels. Some of these results have been briefly described in Carpenter *et al.* (1988b).

MATERIALS AND METHODS

Messenger RNA was prepared from the cerebral cortex of Sprague–Dawley rats of various embryonic and postnatal ages as described previously (Carpenter *et al.*, 1988a). The rats were placed together overnight (10–12 hr) and female rats which were sperm positive were considered pregnant; this day was called Embryonic Day 1 (E1). Embryonic tissue was taken at E15 and E18, and postnatal tissue was taken from newborn (P0), Postnatal Day 5, 10, 15, and 30 (P5, P10, P15, P30), and adult (3–4 month; 200–280 g) animals. In almost all of

[1] Present address: Department of Anatomy, Colorado State University, Fort Collins, Colorado, 80523.

0012-1606/90 $3.00

these cases only the cerebral cortex was removed. The exceptions were the embryonic brains where, because of the small amount of tissue, the entire cerebral hemispheres were sometimes taken. The E15 preparation and one of the three replicate E18 preparations were made from the cerebral hemispheres. Dissections took less than 5 min and the tissue was placed immediately in liquid nitrogen and stored at −70°C until used.

Poly(A)$^+$ mRNA was then isolated from the brain tissue, by phenol/chloroform extraction, precipitation of the total RNA with ethanol, and isolating the poly(A)$^+$ mRNA using oligo(dT) chromatography (Miledi and Sumikawa, 1982; Carpenter *et al.*, 1988a). The amount of RNA obtained was quantified using the optical density reading at 260 nm (OD 1.0 = 40 μg/ml). Poly(A)$^+$ mRNA was dissolved in sterile water, aliquoted, and stored at −70°C until used. When mentioned, mRNA preparations were enriched in mRNA encoding a specific receptor by size fractionation on linear sucrose gradients (Sumikawa *et al.*, 1984, 1989).

Oocytes of *Xenopus laevis* were injected with about 40 ng of mRNA and incubated in Barth's medium at 16–18°C for 4–8 days. Some oocytes were treated with collagenase to remove the enveloping follicular cells 2–4 days before recording (Kusano *et al.*, 1982; Miledi and Parker, 1984; Sumikawa *et al.*, 1989). The oocytes were then voltage-clamped using a two-electrode voltage clamp and agonist-induced membrane currents were recorded at a clamp potential of −60 mV (Kusano *et al.*, 1982; Miledi, 1982; Sumikawa *et al.*, 1989). Ringer's solution (115 m*M* NaCl, 2 m*M* KCl, 1.8 m*M* $CaCl_2$, 5 m*M* Hepes, pH 7.0) was continually superfused over the oocyte and drugs were applied via this perfusate. The concentrations of agonists (100 μ*M* kainate, 1 m*M* glutamate, 100 μ*M* aspartate, 100 μ*M* ACh, 10 μ*M* 5-HT) were sufficient to evoke near maximal responses (Gundersen *et al.*, 1983a, 1984a,c; Parker *et al.*, 1985). Glutamate was applied without glycine or Mg^{2+} added to the Ringer's, but aspartate was applied together with 100 μ*M* glycine.

Sodium and calcium-activated transient outward (T_{out}) currents were evoked by voltage-clamping oocytes at −100 mV and briefly stepping the oocyte to less negative potentials. Sodium currents were obtained by first stepping the oocyte to −20 mV and then subtracting this record from that elicited by the same depolarization in the presence of tetrodotoxin (TTX; 300 n*M*), by the use of a digital oscilloscope (Nicolet). Therefore, the resulting traces (like those seen in Fig. 6) show TTX-sensitive currents, after cancellation of capacitive and passive currents (Parker and Miledi, 1987; Parker *et al.*, 1988). Because of limitations of the voltage clamp, the currents during the first 2–4 msec following a voltage step were not well resolved, and the peak sizes of the sodium currents may thus have been underestimated. However, because the characteristics of the clamp were maintained constant, this should not have introduced any consistent bias between oocytes injected with different mRNA preparations. T_{out} currents were evoked by stepping the oocyte from −100 to 0 mV for about 3 sec, with the oocyte bathed in normal (1.8 m*M*) calcium Ringer's solution (Miledi, 1982). Measurements were made from the peak transient current to the steady-state current elicited by the pulse.

RESULTS

Each oocyte was injected with a roughly constant amount (40 ng) of mRNA, and the translational activities of the various preparations were similar. Thirteen of the 21 preparations used were tested for translational activity *in vitro* using a reticulocyte lysate system (Pelham and Jackson, 1976) and all showed activity within 24% of the mean. The mRNA preparations used for the experiments described here correspond to those used in Carpenter *et al.* (1988a); further details concerning mRNA yields and translational activity are available in that paper—but note that mRNA yields were calculated from OD measurements assuming a factor of 50 μg/ml/unit OD, and not 40 μg/ml as used here.

mRNAs Coding for Excitatory Amino Acid Receptors at Different Ages

Native *Xenopus* oocytes (i.e., noninjected oocytes) do not show substantial membrane current responses to glutamate and other "excitatory" amino acids, but sensitivity can be induced following injection of mRNA from adult rat brain (Gundersen *et al.*, 1984a; Houamed *et al.*, 1984; Smart *et al.*, 1987; Sugiyama *et al.*, 1987; Verdoorn *et al.*, 1987; Fong *et al.*, 1988; Kushner *et al.*, 1988) or human fetal cerebral cortex (Gundersen *et al.*, 1984b). Several responses were induced, preferentially activated by different agonists.

The kainate responses induced by mRNA from each of the different ages were smooth inward currents (at −60 mV; Fig. 1), similar to those seen previously (Gundersen *et al.*, 1984b). Figure 2 (upper panel) shows mean values of the peak current size from different ages. Oocytes injected with mRNA from E15, E18, or newborn animals showed small (less than 20 nA) or no responses to kainate. However, the kainate response increased sharply with development between P5 and P15, reaching adult levels between P15 and P30.

To selectively activate NMDA (*N*-methyl-D-aspartate) receptors, we applied 100 μ*M* aspartate together with 100 μ*M* glycine (Verdoorn *et al.*, 1987; Miledi *et al.*, 1989). This evoked small, smooth inward currents in-

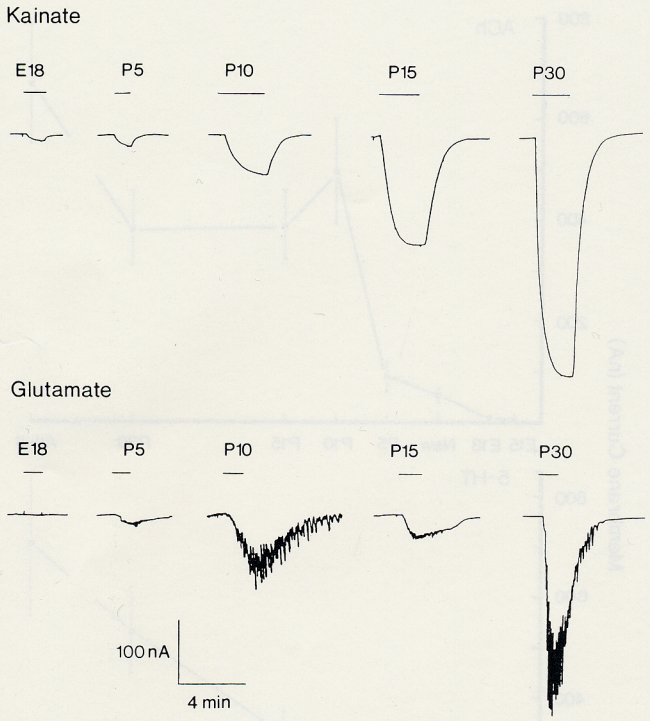

FIG. 1. Membrane currents elicited by 100 μM kainate and 1 mM glutamate in oocytes injected with poly(A)$^+$ mRNAs from different ages of rat cerebral cortex. In this, and other figures, downward deflections indicate inward currents, and drugs were applied via bath perfusion for the times indicated by the bars. Recordings were made at a clamp potential of −60 mV.

jected with mRNA from all ages, and the concentrations used were sufficient to give maximal responses. Application of aspartate alone (100 μM) gave no clear response. The mean current evoked by aspartate plus glycine in oocytes injected with mRNA from E18 animals was 1.9 ± 0.7 nA (5 oocytes), and this increased to 5.7 ± 0.5 nA (7 oocytes) with newborn mRNA and to 8.5 ± 1.3 nA (11 oocytes) with mRNA from adult animals.

At all ages the glutamate-evoked response comprised two components (Fig. 1), a smooth current and an oscillatory chloride current which was mediated by phosphoinositide signalling (Gundersen *et al.*, 1984a; Sumikawa *et al.*, 1984; Parker *et al.*, 1987; Sugiyama *et al.*, 1987; Oosawa and Yamagishi, 1989). The sizes of both components increased with age, but we were unable to separately measure the size of each. Instead, the data in Fig. 2 give the total peak current evoked by glutamate. The peak current size again increased with age, but showed an increase more gradual than that of the kainate response (Fig. 2). Oocytes injected with mRNA from E15 and E18 animals showed little or no response (less than 5 nA), but responses increased with further development, reaching a maximum at about P30 (Figs. 1 and 2).

mRNAs Coding for Serotonin and ACh Receptors at Various Ages

Native oocytes sometimes possess muscarinic receptors, but only rarely show appreciable responses to serotonin (Kusano *et al.*, 1977, 1982). However, sensitivity to both ACh and serotonin is consistently induced following injection of oocytes with brain mRNA (Gundersen *et al.*, 1983a; Sumikawa *et al.*, 1984). Although these responses show smooth and oscillatory components (Gundersen *et al.*, 1987), the predominant responses to both agonists are oscillatory chloride currents, which arise through activation of a phosphoinositide/calcium signalling pathway (Parker *et al.*, 1985; Oron *et al.*, 1985; Parker *et al.*, 1987; Takahashi *et al.*, 1987). In the present

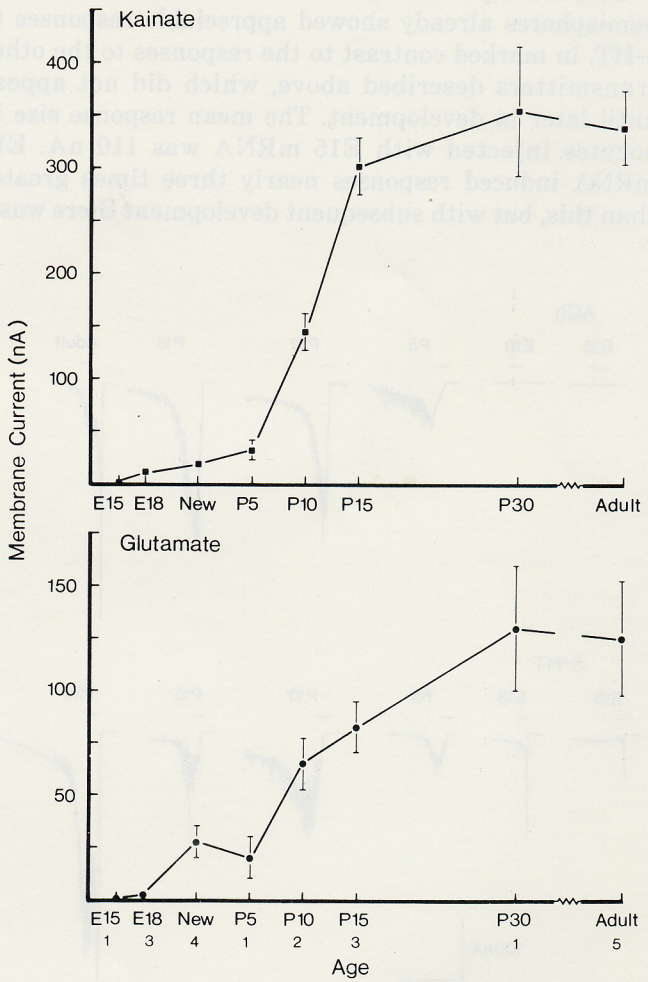

FIG. 2. Mean values of peak currents induced by kainate and glutamate in oocytes injected with mRNAs from various ages. The peak sizes of membrane currents were measured from records like those in Fig. 1 in response to 100 μM kainate or 1 mM glutamate. Each point represents the mean ± SE of 11–66 oocytes (except for E15 glutamate which is only 2 oocytes) from 2 to 13 donors. The number of replicate mRNA preparations is indicated below each age.

experiments, only those donors which yielded oocytes showing little or no native responses to ACh were used. Thus, the sizes of the ACh-evoked currents reflect only the sizes of induced responses.

Oocytes injected with mRNAs from different ages showed wide variation in the size of responses evoked by 5-HT and ACh, but the currents were always of an oscillatory nature (Fig. 3), resembling the response induced by adult brain mRNA. Figure 4 shows the mean peak currents induced at each age. Responses to ACh were not observed in E15 injected oocytes and were small in oocytes injected with E18 and newborn mRNA (less than 60 nA, or 8% of the maximum). The response size increased sharply between P5 and P10 to 66% of the maximum response and finally increased again between P30 and adult injected oocytes (Fig. 4).

Oocytes injected with mRNA from E15 cerebral hemispheres already showed appreciable responses to 5-HT, in marked contrast to the responses to the other transmitters described above, which did not appear until later in development. The mean response size in oocytes injected with E15 mRNA was 110 nA. E18 mRNA induced responses nearly three times greater than this, but with subsequent development there was a slight decrease until about P10, after which the responses grew steadily to a maximum in adult injected oocytes (Figs. 3 and 4).

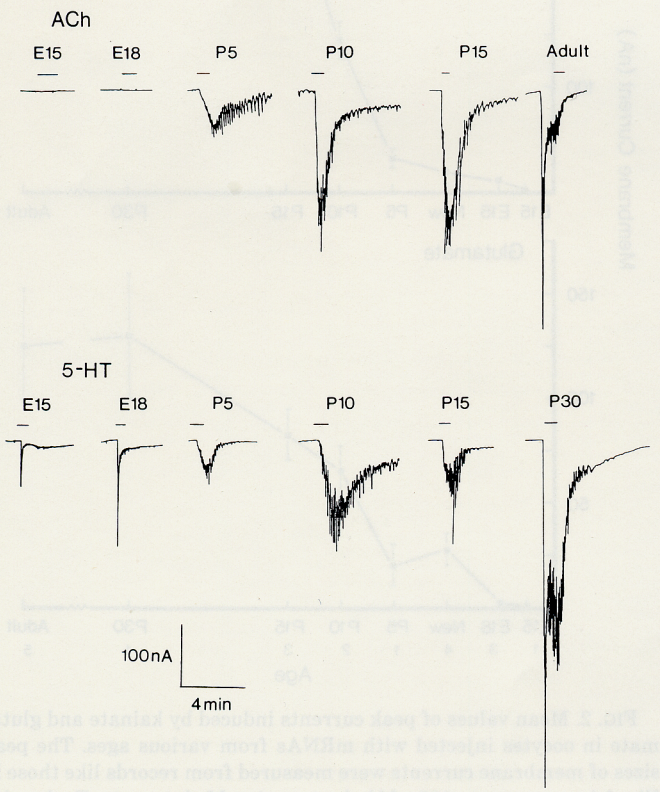

FIG. 3. Membrane currents elicited by ACh (100 μM) or 5-HT (10 μM) in oocytes injected with mRNAs from different ages of rat cerebral cortex. Recordings were made at a clamp potential of −60 mV.

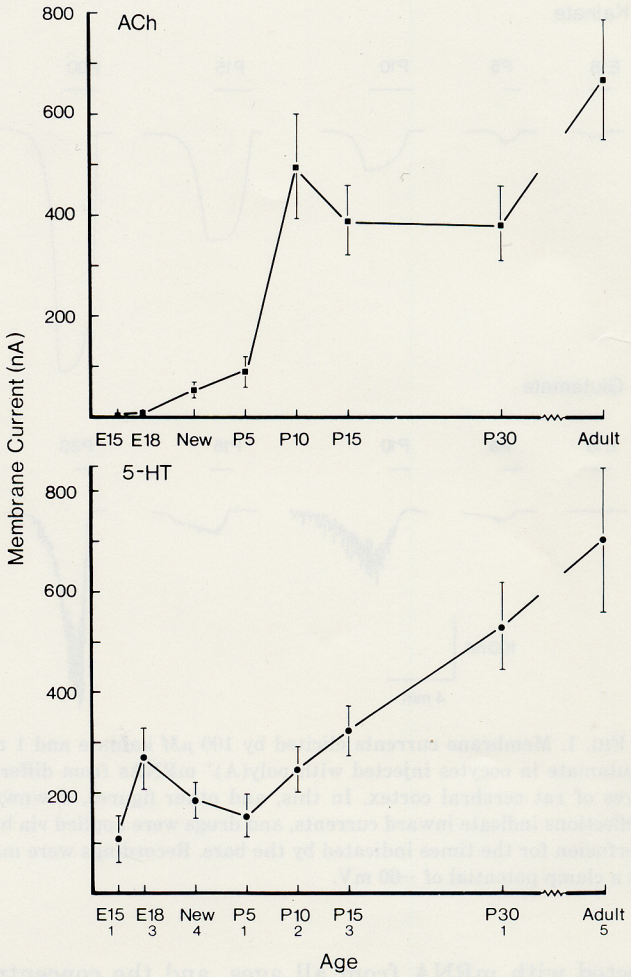

FIG. 4. Mean values of peak responses elicited by ACh (100 μM) or serotonin (10 μM) in oocytes injected with mRNAs from various ages of rat at different developmental stages. The peak membrane currents were measured from records like those in Fig. 3. Each point represents the mean ± SE of 10–54 oocytes from 3 to 12 donors. The number of replicate mRNA preparations is indicated below each age.

The responses to ACh and 5-HT showed a complex time course, typically comprising an initial "spike" of current, followed by a gradually increasing and decreasing sequence of oscillations. However, there was great variability between different oocytes, and often either the spike or oscillatory components were small or absent. Each of these components increased with age, but so far we do not know if they increased at different rates.

Reversal Potentials and Antagonists

The reversal potential of the kainate-activated current was determined by stepping the clamp potential to

various levels before and during the application of kainate. Kainate-activated currents measured in Fig. 5 show current/voltage relationships for oocytes injected with E18, P5, or adult mRNAs. Oocytes injected with E15 mRNA gave responses too small to obtain reliable data. There were no obvious changes in reversal potential across ages (E18 to adult), suggesting a similar ionic basis for the currents mediated by channels expressed by mRNAs from different ages. The mean reversal potential, pooling all oocytes, was -4.8 ± 2.7 mV (SE, 13 oocytes, 7 ages). Furthermore, within the voltage range examined, the currents in all cases varied linearly with potential, indicating that the gating of the kainate-activated channel is not markedly affected by voltage.

With mRNAs from all ages, the ACh- and 5-HT-induced oscillatory currents decreased when the cell was depolarized and inverted direction at about −25 mV, the chloride equilibrium potential in *Xenopus* oocytes (Kusano *et al.*, 1982), suggesting that they are carried largely by a flux of chloride ions. This is expected, since the oscillatory currents mediated by ACh and 5-HT receptors are thought to arise through the opening of endogenous calcium-activated chloride currents in the oocyte membrane (Parker *et al.*, 1985; Oron *et al.*, 1985; Parker *et al.*, 1987; Takahashi *et al.*, 1987). However, small, smooth currents sometimes remained when oocytes were clamped to potentials near the chloride equilibrium potential, suggesting the existence of currents with other ionic bases (Gundersen *et al.*, 1987; Parker *et al.*, 1989). Developmental changes in these components remain to be studied.

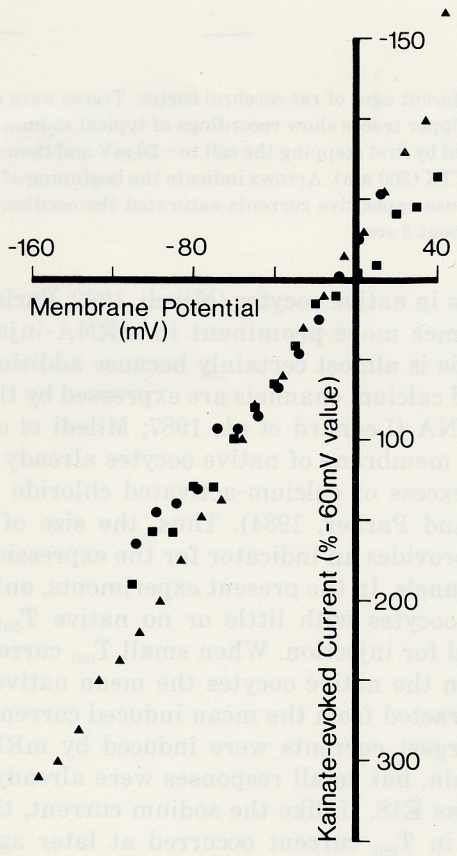

FIG. 5. Current/voltage relations of kainate responses from three oocytes injected with either E18 (●), P5 (■), or adult (▲) mRNA. Points indicate the current induced at each potential by 100 μM kainate after subtraction of passive membrane currents evoked by the same voltage steps in the absence of kainate. Values are expressed as a percentage of the kainate-induced current at a holding potential of −60 mV.

In oocytes injected with newborn, P10, P15, and adult mRNAs, the induced ACh (100 μm) response was completely blocked by atropine (1 μm), indicating that it was due to activation of muscarinic receptors. Earlier ages were not tested with atropine because the ACh-induced response was too small to obtain reliable data. There was no evidence for the presence of nicotinic receptors. The 5-HT (10 μm) response was completely blocked by methysergide (1 μm) in all ages (E15 through adult).

mRNAs Coding for Voltage-Operated Channels at Various Ages

As well as causing the expression of exogenous neurotransmitter receptors, mRNA from brain induced the appearance of several voltage-activated membrane channels (Gundersen *et al.*, 1983b; Leonard *et al.*, 1987; Sigel, 1987). Two different induced currents were examined, a rapidly inactivating sodium current and a calcium-activated outward transient (T_{out}) current carried by chloride ions.

In oocytes injected with mRNA from adult brain the sodium current is a transient inward current which appears when the cell is depolarized to potentials beyond about −40 mV (Gundersen *et al.*, 1983b; Sigel, 1987). In oocytes injected with mRNA from different ages of rat cerebral cortex the sodium current evoked was similar to that seen previously in oocytes injected with mRNA from adult rat brain and fetal human brain (Gundersen *et al.*, 1983b, 1984b). Because of difficulties in rapidly clamping the cell, measurements were made at a potential (−20 mV) at which the sodium current inactivates relatively slowly. Even so, the peak values of the sodium currents were almost certainly underestimated, but the comparisons between mRNAs should still be valid. At all ages, the sodium current was blocked by TTX and the measurements presented are of the TTX-sensitive current (as described under Materials and Methods). We did not detect any TTX-insensitive currents, but small (less than about 25 nA) currents would not have been seen in the presence of large capacitive currents.

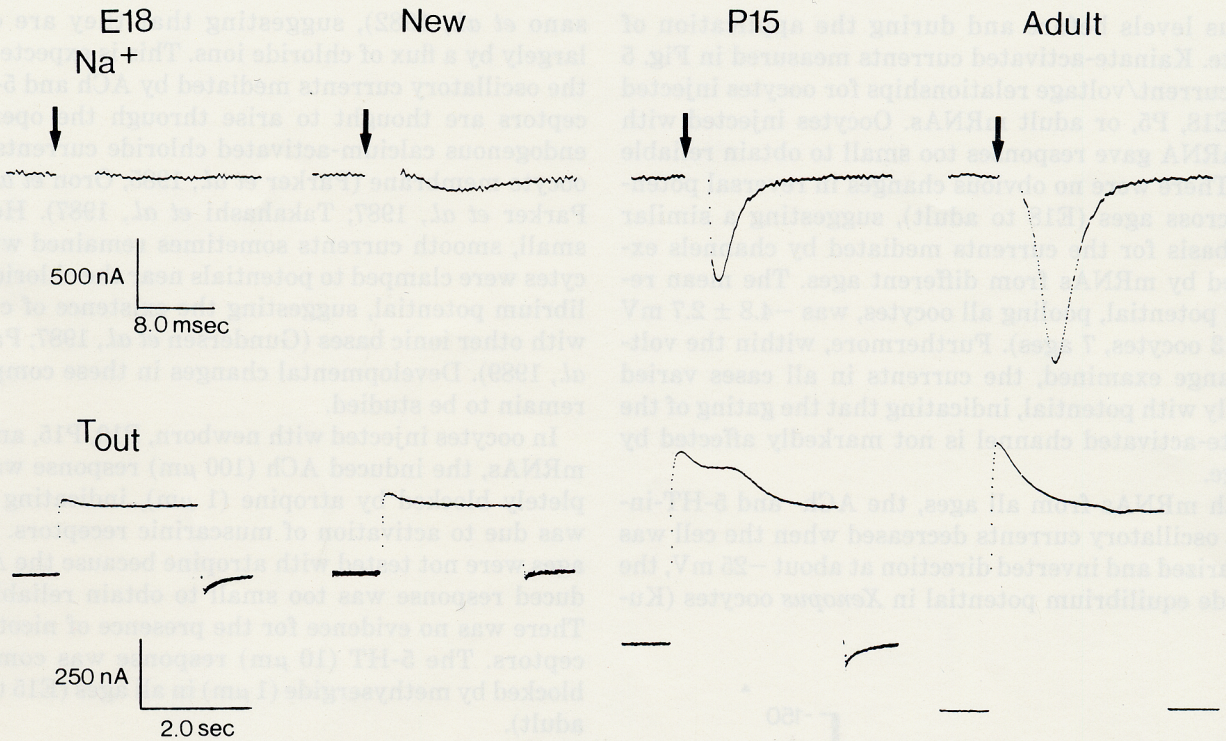

FIG. 6. Sodium and T_{out} currents evoked in oocytes injected with mRNAs from different ages of rat cerebral cortex. Traces were obtained by holding the oocyte at −100 mV and briefly stepping the potential to various levels. Upper traces show recordings of typical sodium currents in oocytes injected with mRNAs from various ages. In all cases, the traces were obtained by first stepping the cell to −20 mV and then subtracting from that record the current elicited by the same depolarization in the presence on TTX (300 nm). Arrows indicate the beginning of the voltage step. Traces are blanked out during the first few msec after the voltage step because capacitive currents saturated the oscilloscope. Lower traces show T_{out} currents obtained by stepping the oocyte from −100 to 0 mV for about 3 sec.

Like the ligand-gated responses, the size of the voltage-activated sodium current increased with age (Figs. 6 and 7). Oocytes injected with E15 mRNA showed little or no detectable current, but the size of the sodium current subsequently increased steeply after E18 and reached a maximum between P10 and P15.

In addition to measuring the size of the sodium current, we also studied its rate of inactivation. The half-decay time of the sodium current in oocytes injected with mRNA from newborn cerebral cortex was 4.70 ± 0.42 msec (13 oocytes, four donors) and shortened to 2.73 ± 0.16 msec (19 oocytes, three donors) in oocytes injected with mRNA from adult cerebral cortex. For both ages measurements were taken from oocytes injected with whole mRNA and with fractionated mRNAs which were concentrated in species coding for the sodium channel. There was no apparent difference in the rate of inactivation between the whole mRNAs and the fractions.

Depolarization of oocytes to around 0 mV also evokes T_{out} current, which is carried by chloride ions and is dependent on the influx of calcium ions. This current is much slower than the sodium current, so the two currents can be easily discriminated. The T_{out} current is often seen in native oocytes (Miledi, 1982; Barish, 1983), but becomes more prominent in mRNA-injected oocytes. This is almost certainly because additional voltage-gated calcium channels are expressed by the exogenous mRNA (Leonard *et al.*, 1987; Miledi *et al.*, 1986), since the membrane of native oocytes already contains a great excess of calcium-activated chloride channels (Miledi and Parker, 1984). Thus, the size of the T_{out} current provides an indicator for the expression of calcium channels. In the present experiments, only donors yielding oocytes with little or no native T_{out} current were used for injection. When small T_{out} currents were present in the native oocytes the mean native current was subtracted from the mean induced current.

The largest currents were induced by mRNA from adult brain, but small responses were already present as early as E18. Unlike the sodium current, the major increase in T_{out} current occurred at later ages, after about P5 (Figs. 6 and 7).

DISCUSSION

In this work we measured membrane currents evoked by various neurotransmitters, or by voltage steps, in *Xenopus* oocytes injected with mRNA obtained from rat

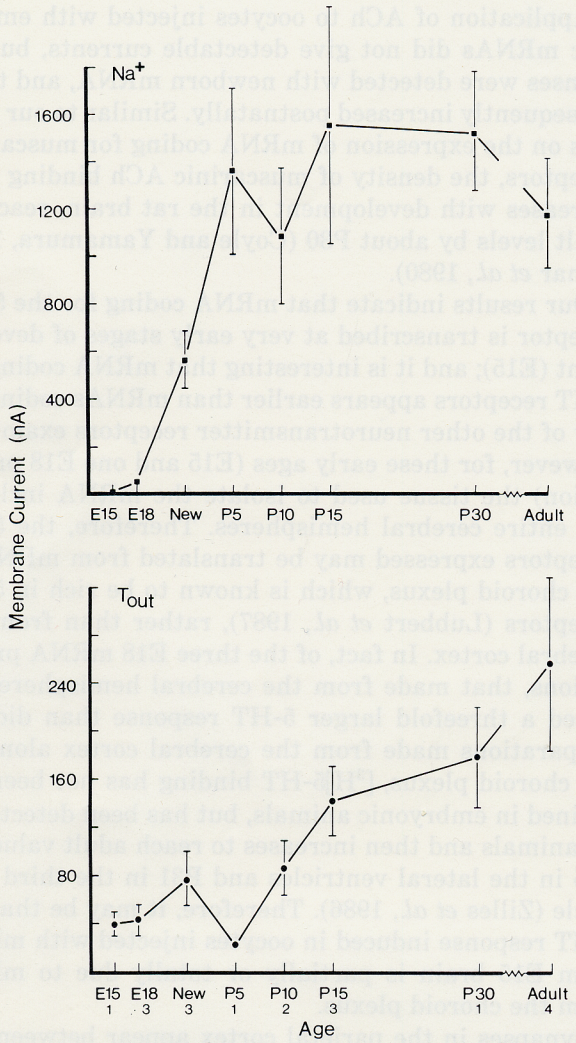

FIG. 7. Mean sizes of sodium and T_{out} currents. Measurements were obtained from records like those in Fig. 6, by holding the cell at −100 mV and stepping the oocyte to either −20 mV for the sodium current or to 0 mV for the T_{out} current. Each point is the mean ± SE of 3–35 oocytes from two to six donors. The number of replicate mRNA preparations is indicated below each age.

brains at different developmental stages. Since each oocyte was injected with an approximately constant amount of mRNA, the simplest interpretation of the results is that changes in the size of a particular response induced by mRNA from different ages reflect changes in the proportion of mRNA coding for that channel or receptor. A great advantage of this technique is that it allows the study of receptors and channels for which specific probes are not yet available. However, several limitations must be acknowledged. One is that, because mRNA was extracted from a large brain region, the sizes and properties of expressed responses will represent a weighted average of those in all cells. Changes in mRNA levels in a minor cell population might, therefore, escape detection. Second, as we have discussed before (Carpenter *et al.*, 1988a), there may be factors in addition to the amount of mRNA which affect the size of the induced response. Some of these can already be discounted. For example, changes in the ionic basis of the responses could alter their size when measured at a fixed potential, but this could be ruled out for many of the currents examined, since we found no differences in reversal potentials across development. However, other possibilities remain, including changes in the lifetime or conductance of the membrane channels, differences in efficiency of translation of different messengers, and the expression in the oocyte of proteins which regulate the expression or function of the channels and receptors. Finally, a separate issue is that the expression of functional receptors and channels in neurons is likely to be regulated not only at the transcriptional level, but also at translational and post-translational levels. Thus, the appearance of receptors and channels in neurons might not correspond directly with the relative amounts of mRNA which encode them. For all these reasons, we were therefore interested in comparing our results with what is known from other techniques about the expression of receptors and channels in the developing brain.

mRNAs Coding for Neurotransmitter Receptors

L-Glutamate and L-aspartate are thought to be the major excitatory neurotransmitters in the central nervous system and activate a variety of receptors (Monaghan *et al.*, 1989). In oocytes injected with brain mRNA, several excitatory amino acid receptors are expressed. As mentioned previously, application of glutamate induces a response which has a smooth component and an oscillatory component (Gundersen *et al.*, 1984a; Houamed *et al.*, 1984; Sugiyama *et al.*, 1987). Application of kainate induces a smooth, nondesensitizing inward current (Gundersen *et al.*, 1984a), which arises through activation of a specific receptor type (Monaghan *et al.*, 1989). In addition, quisqualate, *N*-methyl-D-aspartate, and other specific glutamate receptor agonists have also been shown to induce responses in mRNA-injected oocytes (Gundersen *et al.*, 1984a; Verdoorn *et al.*, 1987; Kushner *et al.*, 1988).

Responses to kainate were small with mRNAs from ages earlier than about P5, but then grew steeply to reach adult levels by P15. Expression of NMDA receptors also increased with age but, because of the small sizes of the induced currents, we were unable accurately to follow their development. Glutamate activates several types of receptors, and results obtained using this

agonist (Fig. 2, lower panel) are thus more difficult to interpret. However, the small sizes of currents mediated by NMDA receptors indicates that these receptors could not have contributed appreciably to the glutamate response. Furthermore, a large part of the response to glutamate was an oscillatory chloride current, which is mediated by a class of quisqualate receptors which stimulate phosphoinositide signalling (Parker *et al.*, 1987; Sugiyama *et al.*, 1987; Oosawa and Yamagishi, 1989). The endogenous phosphoinositide pathway in the oocyte is able to generate large chloride currents (Kusano *et al.*, 1982; Miledi and Parker, 1984), so that the induction of oscillatory responses to glutamate almost certainly reflects the expression of exogenous receptors, and not the expression of additional chloride channels or other components of the signalling pathway. We thus conclude that expression of the mRNA encoding the phosphoinositide-linked quisqualate receptor is low before E18 and then increases gradually to reach a maximal level by about P30.

The developmental appearance of glutamate receptors in the rat brain has been studied by examining the appearance of [^{3}H]glutamate binding to sodium-independent sites. The binding to these sites in the cerebral cortex was found to increase postnatally, reaching adult levels by P50 (Sanderson and Murphy, 1982). However, interpretation of these results has been recently complicated by the finding of a chloride-dependent glutamate uptake system (Bridges *et al.*, 1987; Recasens *et al.*, 1987), so that the sodium-independent sites seen previously may include chloride-dependent uptake sites as well as glutamate receptors. The ontogeny of kainate receptors has not been studied in the cerebral cortex. However, [^{3}H]kainate binding has been examined in the developing rat cerebellum (Slevin and Coyle, 1981) and the developing rat and human hippocampi (Represa *et al.*, 1986). In all these cases, binding was found to increase with age. Therefore, at least qualitatively, the findings from the binding studies agree with our findings.

Although there are two major types of ACh receptor in the CNS, muscarinic and nicotinic, only muscarinic receptors were expressed by mRNA from any of the developmental stages, since the responses were completely blocked by atropine. The muscarinic receptors generate membrane currents by acting through the phosphoinositide pathway endogenous to the oocyte, and this is also the case for serotonergic receptors which evoke an oscillatory current (Parker *et al.*, 1987). As discussed above, changes in expression of responses to ACh and serotonin almost certainly reflect changes in expression of the respective receptors, and not of the ion channels which they activate.

Application of ACh to oocytes injected with embryonic mRNAs did not give detectable currents, but responses were detected with newborn mRNA, and these subsequently increased postnatally. Similar to our findings on the expression of mRNA coding for muscarinic receptors, the density of muscarinic ACh binding sites increases with development in the rat brain, reaching adult levels by about P30 (Coyle and Yamamura, 1976; Kuhar *et al.*, 1980).

Our results indicate that mRNA coding for the 5-HT receptor is transcribed at very early stages of development (E15); and it is interesting that mRNA coding for 5-HT receptors appears earlier than mRNAs coding for any of the other neurotransmitter receptors examined. However, for these early ages (E15 and one E18 preparation) the tissue used to isolate the mRNA included the entire cerebral hemispheres. Therefore, the 5-HT receptors expressed may be translated from mRNA in the choroid plexus, which is known to be rich in 5-HT receptors (Lubbert *et al.*, 1987), rather than from the cerebral cortex. In fact, of the three E18 mRNA preparations, that made from the cerebral hemispheres induced a threefold larger 5-HT response than did the preparations made from the cerebral cortex alone. In rat choroid plexus, [^{3}H]5-HT binding has not been examined in embryonic animals, but has been detected in P1 animals and then increases to reach adult values by P25 in the lateral ventricles and P31 in the third ventricle (Zilles *et al.*, 1986). Therefore, it may be that the 5-HT response induced in oocytes injected with mRNA from E15 brain is partially or totally due to mRNA from the choroid plexus.

Synapses in the parietal cortex appear between P15 and P30 (Aghajanian and Bloom, 1967), while we find that the mRNAs coding for kainate, glutamate, and ACh receptors appear between newborn and P5, and mRNA coding for 5-HT receptors appears earlier than E15. Thus, it would appear that neurotransmitter receptors are expressed in the brain before the formation of functional synaptic connections. Other investigators have proposed that 5-HT has a role in the early development of the nervous system (Lauder and Bloom, 1975; Raedler and Raedler, 1978; Lauder and Krebs, 1978; Lauder *et al.*, 1981; Haydon *et al.*, 1984; Chubakov *et al.*, 1986). 5-HT responses are present in oocytes injected with E18 cerebral cortex mRNA, which seems to be in accord with these proposals. However, it remains possible that although mRNAs coding for receptors have been translated in the oocyte, they may not necessarily be translated in the rat brain. Therefore, when the appropriate probes become available it would be interesting to complement these studies with binding and *in situ* hybridization studies to determine which of the

neurons in the cerebral cortex contain mRNAs for these receptors and whether the mRNAs are in fact translated in the developing brain.

mRNAs Coding for Voltage-Operated Channels

The TTX-sensitive sodium current was quite small in oocytes injected with E18 mRNA and subsequently increased steeply, reaching adult values between P5 and P15 (Fig. 7). Saxitoxin (STX) and TTX are both specific blockers of voltage-sensitive sodium channels and have been used in radioligand binding experiments to identify sodium channels in the developing rat brain. STX binding was detected in E19 whole brain and increased rapidly with age until P15 when a second, more gradual increase appeared until adult levels were reached at 4.5 weeks after birth (Baumgold *et al.*, 1983; Baumgold, 1985). Similarly, TTX binding appeared at E17 in rat brains and increased postnatally, reaching a plateau by P20 (Lombet *et al.*, 1983). These results are in agreement with our findings in that the binding levels increase at approximately the same time as the mRNAs coding for the TTX-sensitive sodium channels.

The inactivation of the sodium current appeared to become faster with age. Although it has been recently shown that mRNA species not present in the high-molecular fraction can influence the inactivation process of rat sodium channels in oocytes (Krafte *et al.*, 1988) we did not detect differences between the oocytes injected with fractionated mRNA and whole mRNA.

The expression of voltage-gated calcium channels could be detected in the oocyte with mRNA from ages as early as E15, and the expression increased progressively with age. The developmental appearance of calcium channels has been studied in the rat and mouse brain using labeled nitrendipine (Matsubayashi *et al.*, 1984; Kazazoglou *et al.*, 1983; Erman *et al.*, 1983) and (−)desmethoxyverapamil (Mourre *et al.*, 1987). These studies showed binding in late fetal stages (Matsubayashi *et al.*, 1984) which increased postnatally to reach adult levels between P15 (Kazazoglou *et al.*, 1983) and P28 (Matsubayashi *et al.*, 1984). Thus, the levels of mRNA coding for calcium channels seem to roughly parallel the number of calcium channels detected in brains at different developmental stages by ligand binding, but the interpretation may be complicated by the existence of several subtypes of calcium channels, with different binding properties and electrophysiological properties (Tsien *et al.*, 1988).

In conclusion, we have demonstrated the use of the *Xenopus* oocyte expression system to study the developmental expression of mRNAs encoding a variety of receptors and channels. This approach is particularly useful for studying receptors for which specific probes are not available. Furthermore, it gives information about not only the amount of mRNA present, but also about the functional properties of the channels and receptors. So far, we have found few developmental changes in the properties of the responses described here, but it remains possible that a more detailed characterization may reveal differences.

This work was supported by grants (NS23284) from the U.S. Public Health Services and (BNS-8513515) from NSF. M.K.C. was supported by a training grant (MH141599) and a fellowship (MH09633) from NIMH.

REFERENCES

AGHAJANIAN, G. K., and BLOOM, F. E. (1967). The formation of synaptic junctions in the developing rat brain: A quantitative electron microscopic study. *Brain Res.* **6,** 716–727.

AKAGI, H., and MILEDI, R. (1988). Expression of glycine and other amino acid receptors by rat spinal cord mRNA in *Xenopus* oocytes. *Neurosci. Lett.* **95,** 262–268.

BARISH, M. E. (1983). A transient calcium-dependent chloride current in the immature *Xenopus* oocyte. *J. Physiol.* **342,** 309–325.

BAUMGOLD, J. (1985). Changes in the ratio of two separate toxin binding sites on the sodium channel protein during rat brain development. *Dev. Brain Res.* **17,** 271–274.

BAUMGOLD, J., ZIMMERMAN, I., and BAMBRICK, L. (1983). Appearance of [^{3}H]saxitoxin binding sites in the developing rat brain. *Dev. Brain Res.* **9,** 405–407.

BRIDGES, R. J., NEITO-SAMPEDRO, M., KADRI, M., and COTMAN, C. (1987). A novel chloride-dependent L-[^{3}H]glutamate binding site in astrocyte membranes. *J. Neurosci.* **48(6),** 1709–1715.

CARPENTER, M. K., PARKER, I., and MILEDI, R. (1988a). Expression of GABA and glycine receptors by messenger RNAs from the developing rat cortex. *Proc. R. Soc. London B* **234,** 159–170.

CARPENTER, M. K., PARKER, I., and MILEDI, R. (1988b). Changes in messenger RNAs coding for neurotransmitter receptors in the developing rat cerebral cortex. *Soc. Neurosci. Abstr.* **14,** 414.

CHUBAKOV, A. R., GROMOVA, E. A., KONOCLAV, G. V., SARKISOVA, E. F., and CHUMASOV, E. I. (1986). The effects of serotonin on the morpho-functional development of rat cerebral neocortex in tissue culture. *Brain Res.* **369,** 285–297.

COYLE, J. T. (1977). Biochemical aspects of neurotransmitters in the developing brain. *Int. Rev. Neurobiol.* **20,** 65–103.

COYLE, J. T. (1982). Development of neurotransmitters in the neocortex. *Neurosci. Res. Prog. Bull.* **20,** 479–492.

COYLE, J. T., and YAMAMURA, H. I. (1976). Neurochemical aspects of the ontogenesis of cholinergic neurons in the rat brain. *Brain Res.* **118,** 429–440.

DASCAL, N. (1987). The use of *Xenopus* oocytes for the study of ion channels. *CRC Crit. Rev. Biochem.* **22,** 317–387.

ERMAN, R. D., YAMAMURA, H. I., and ROESKE, W. R. (1983). The ontogeny of specific binding sites for the calcium channel antagonist, nitrendipine, in mouse heart and brain. *Brain Res.* **278,** 327–331.

FONG, T. M., DAVIDSON, N., and LESTER, H. (1988). Properties of two classes of rat brain acidic amino acid receptors induced by distinct mRNA populations in *Xenopus* oocytes. *Synapse* **2,** 657–665.

GUNDERSEN, C. B., MILEDI, R., and PARKER, I. (1983a). Serotonin receptors induced by exogenous messenger RNA in *Xenopus* oocytes. *Proc. R. Soc. London B* **219,** 103–109.

GUNDERSEN, C. B., MILEDI, R., and PARKER, I. (1983b). Voltage-operated channels induced by foreign messenger RNA in *Xenopus* oocytes. *Proc. R. Soc. London B* **220,** 131–130.

GUNDERSEN, C. B., MILEDI, R., and PARKER, I. (1984a). Glutamate and kainate receptors induced by rat brain mRNA in *Xenopus* oocytes. *Proc. R. Soc. London B* **221,** 127–243.

GUNDERSEN, C. B., MILEDI, R., and PARKER, I. (1984b). Messenger RNA from human brain induces drug- and voltage-operated channels in *Xenopus* oocytes. *Nature (London)* **308,** 421–424.

GUNDERSEN, C. B., MILEDI, R., and PARKER, I. (1984c). Properties of human glycine receptors expressed in *Xenopus* oocytes. *Proc. R. Soc. London B* **221,** 235–244.

GUNDERSEN, C. B., MILEDI, R., and PARKER, I. (1987). Serotonin receptors expressed by brain mRNA in *Xenopus* oocytes mediate three different ionic currents. *J. Physiol.* **386,** 83P.

HAYDON, P. G., MCCOBB, D. P., and KATER, S. B. (1984). Serotonin selectively inhibits growth cone motility and synaptogenesis of specific identified neurons. *Science* **226,** 561–564.

HOUAMED, K. M., BILVE, G., SMART, T. G., CONSTANTI, A., BROWN, D. A., BARNARD, E. A., and RICHARDS, B. M. (1984). Expression of functional GABA, glycine and glutamate receptors in *Xenopus* oocytes injected with rat brain mRNA. *Nature (London)* **310,** 318–321.

HUNT, S. P. (1988). The development of neurotransmitter receptors. *In* "The Making of the Nervous System" (J. G. Parnavelas, C. D. Stern, and R. V. Stirling, Eds.), pp. 454–472. Oxford Univ. Press, Oxford.

JOHNSON, M. V. (1985). Neurotransmitters. *In* "Developmental Neurochemistry" (R. C. Wiggins, D. W. McCandless, and S. J. Enna, Eds.), pp. 193–224. Univ. of Texas Press, Austin.

KAZAZOGLOU, T., SCHMID, A., RENAUD, J. F., and LAZDUNSKI, M. (1983). Ontogenic appearance of Ca^{2+} channels characterized as binding sites for nitrendipine during development of nervous, skeletal and cardiac muscle systems in the rat. *FEBS* **164(1),** 75–79.

KRAFTE, D. S., SNUTCH, T. P., LEONARD, J. P., DAVIDSON, N., and LESTER, H. (1988). Evidence for the involvement of more than one mRNA species in controlling the inactivation process of rat and rabbit brain Na channels expressed in *Xenopus* oocytes. *J. Neurosci.* **8(8),** 2859–2868.

KUHAR, M. J., BIRDSALL, N. J. M., BURGEN, A. S. V., and HULME, E. C. (1980). Ontogeny of muscarinic receptors in rat brain. *Brain Res.* **184,** 375–383.

KUSANO, K., MILEDI, R., and STINNAKRE, J. (1977). Acetylcholine receptors in the oocyte membrane. *Nature (London)* **270,** 739–741.

KUSANO, K., MILEDI, R., and STINNAKRE, J. (1982). Cholinergic and catecholinergic receptors in the *Xenopus* oocyte membrane. *J. Physiol.* **328,** 143–170.

KUSHNER, L., LERNA, J., ZUKIN, R. S., and BENNET, M. V. L. (1988). Coexpression of NMDA and phenylcyclidine receptors in *Xenopus* oocytes injected with rat brain mRNA. *Proc. Natl. Acad. Sci. USA* **85,** 3250–3254.

LANIER, L. P., DUNN, A. J., and VAN HARTESVELDT, C. (1976). Development of neurotransmitters and their function in the brain. *Rev. Neurosci.* **2,** 195–256.

LAUDER, J. M., and BLOOM, F. E. (1975). Ontogeny of monoamine neurons in the locus coerulus, raphe nuclei and substantia nigra of the rat brain. II. Synaptogenesis. *J. Comp. Neurol.* **163,** 251–264.

LAUDER, J. M., and KREBS, H. (1978). Serotonin as a differentiation signal in early neurogenesis. *Dev. Neurosci.* **1,** 15–30.

LAUDER, J. M., WALLACE, J. A., and KREBS, H. (1981). Roles for serotonin in neuroembryogenesis. *Adv. Exp. Biol. Med.* **133,** 477–506.

LEONARD, J. M., NARGEOT, J., SNUTCH, T. P., DAVIDSON, N., and LESTER, H. (1987). Ca channels induced in *Xenopus* oocytes by adult rat brain mRNA. *J. Neurosci.* **7(3),** 875–881.

LOMBET, A., KAZAZOGLOU, T., DELPONT, E., RENAUD, J-F., and LAZDUNSKI, M. (1983). Ontogenic appearance of Na^+ channels characterized as high affinity binding sites for tetrodotoxin during development of the rat nervous and skeletal muscle systems. *Biochem. Biophys. Res. Commun.* **110(3),** 894–901.

LUBBERT, H., HOFFMAN, B. J., SNUTCH, T. P., VAN DYKE, T., LEVINE, A. J., HARTIG, P. R., LESTER, H. A., and DAVIDSON, N. (1987). cDNA cloning of a serotonin 5-HT_{1C} receptor by electrophysiological assays of mRNA-injected *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* **84,** 4332–4336.

MATSUBAYASHI, H., KITO, S., ITOGA, E., MIZUNO, K., and MIYOSHI, R. (1984). Ontogenetic development of the specific [^{3}H]nitrendipine binding sites in the whole rat brain. *Adv. Exp. Med. Biol.* **175,** 193–204.

MILEDI, R. (1982). A calcium-dependent transient outward current in *Xenopus laevis* oocytes. *Proc. R. Soc. London B* **215,** 491–497.

MILEDI, R., and PARKER, I. (1984). Chloride current induced by injection of calcium into *Xenopus* oocytes. *J. Physiol.* **357,** 173–183.

MILEDI, R., PARKER, I., and SUMIKAWA, K. (1986). Calcium channels expressed in *Xenopus* oocytes by mRNA from rat brain. *J. Physiol.* **374,** 38.

MILEDI, R., PARKER, I., and SUMIKAWA, K. (1989). Transplanting receptors from brains into oocytes. *In* "Fidia Award Lecture Series" (J. Smith, Ed.). Raven Press, New York. Vol. 3, pp. 57–90.

MILEDI, R., and SUMIKAWA, K. (1982). Synthesis of cat muscle acetylcholine receptors by *Xenopus* oocytes. *Biomed. Res.* **3,** 390–399.

MONAGHAN, D. T., BRIDGES, R. J., and COTMAN, C. (1989). The excitatory amino acid receptors: Their classes, pharmacology, and distinct properties in the function of the central nervous system. *Annu. Rev. Pharmacol. Toxicol.* **29,** 365–402.

MOURRE, C., CERVERA, P., and LAZDUNSKI, M. (1987). Autoradiographic analysis in rat brain of the postnatal ontogeny of voltage-dependent Na^+ channels, Ca^{2+}-dependent K^+ channels and slow Ca^{2+} channels identified as receptors for tetrodotoxin, apamin and (−) desmethoxyverapamil. *Brain Res.* **417,** 21–32.

ORON, Y., DASCAL, M., NADLER, E., and LUPU, M. (1985). Inositol 1,4,5-trisphosphate mimics muscarinic response in *Xenopus* oocytes. *Nature (London)* **313,** 141–143.

OOSAWA, Y., and YAMAGISHI, S. (1989). Rat brain glutamate receptors activate chloride channels in *Xenopus* oocytes coupled by inositol triphosphate and Ca^+. *J. Physiol.* **408,** 223–232.

PARKER, I., GUNDERSEN, C. B., and MILEDI, R. (1985). Intracellular Ca^{2+}-dependent and Ca^{2+}-independent responses of rat brain serotonin receptors transplanted to *Xenopus* oocytes. *Neurosci. Res.* **2,** 491–496.

PARKER, I., and MILEDI, R. (1987). Tetrodotoxin-sensitive sodium current in native *Xenopus* oocytes. *Proc. R. Soc. London B* **232,** 289–296.

PARKER, I., PANICKER, M. M., and MILEDI, R. (1989). Serotonin receptors expressed in *Xenopus* oocytes by mRNA from brain mediate a closing of K^+ membrane channels. *Mol. Brain Res.* in press.

PARKER, I., SUMIKAWA, K., GUNDERSEN, C. B., and MILEDI, R. (1988). Expression of ACh-activated channels and sodium channels by messenger RNAs from innervated and denervated muscle. *Proc. R. Soc. London B* **233,** 235–246.

PARKER, I., SUMIKAWA, K., and MILEDI, R. (1985). Messenger RNA from bovine retina induces kainate and glycine receptors in *Xenopus* oocytes. *Proc. R. Soc. London B* **225,** 99–106.

PARKER, I., SUMIKAWA, K., and MILEDI, R. (1987). Activation of a common effector system by different brain neurotransmitter receptors in *Xenopus* oocytes. *Proc. R. Soc. London B* **231,** 37–45.

PELHAM, H. R. B., and JACKSON, R. J. (1976). An efficient mRNA-dependent translation system from reticulocyte lysates. *Eur. J. Biochem.* **67,** 247–256.

RAEDLER, E., and RAEDLER, A. (1978). Autoradiographic study of early neurogenesis in rat neocortex. *Anat. Embryol.* **154,** 267–284.

RECASENS, M., PIN, J. P., and BOCKAERT (1987). Chloride transport blockers inhibit the chloride-dependent glutamate binding to rat brain membranes. *Neurosci. Lett.* **74,** 211–216.

REPRESA, A., TREMBLAY, E., SCHOEVART, D., and BEN-ARI, Y. (1986).

Development of high affinity kainate binding sites in human and rat hippocampi. *Brain Res.* **384,** 170–174.

SANDERSON, C., and MURPHY, S. (1982). Glutamate binding in rat cerebral cortex during ontogeny. *Dev. Brain Res.* **2,** 329–339.

SIGEL, E. (1987). Properties of single sodium channels translated by *Xenopus* oocytes after injection with messenger ribonucleic acid. *J. Physiol.* **386,** 73–90.

SLEVIN, J. T., and COYLE, J. T. (1981). Ontogeny of receptor binding sites for ^{3}H-glutamic acid and ^{3}H-kainic acid in the rat cerebellum. *J. Neurochem.* **37(2),** 531–533.

SMART, T. G., HOUAMED, K. M., VAN RENTERGHAM, C., and CONSTANTI, A. (1987). mRNA-directed synthesis and insertion of functional amino acid receptors in *Xenopus* oocytes. *Biochem. Soc. Trans.* **15,** 117–222.

SUGIYAMA, H., ITO, I., and HIRONO, C. (1987). A new type of glutamate receptor linked to inositol phospholipid metabolism. *Nature (London)* **235,** 531–533.

SUMIKAWA, K., PARKER, I., and MILEDI, R. (1984). Partial purification and functional expression of brain mRNAs coding for neurotransmitter receptors and voltage-operated channels. *Proc. Natl. Acad. Sci. USA* **81,** 7994–7998.

SUMIKAWA, K., PARKER, I., and MILEDI, R. (1989). Expression of neurotransmitter receptors and voltage-activated channels from brain mRNA in *Xenopus* oocytes. *In* "Methods in Neurosciences" (P. H. Conn, Ed.), pp. 30–45. Academic Press, San Diego.

TAKAHASHI, T., NEHER, E., and SAKMAN, B. (1987). Rat brain serotonin receptors in *Xenopus* oocytes are coupled by intracellular calcium to endogenous channels. *Proc. Natl. Acad. Sci. USA* **84,** 5063–5067.

TELANG, S. D., and ENNA, S. J. (1985). Ontogenetic development of CNS neurotransmitter receptors. *In* "Developmental Neurochemistry" (R. C. Wiggins, D. W. McCandless, and S. J. Enna, Eds.), pp. 225–247. Univ. of Texas Press, Austin.

TSIEN, R. W., LIPSCOMBE, D., MADISON, D. V., BLEY, K. R., and FOX, A. P. (1988). Multiple types of neuronal calcium channels and their selective modulation. *TINS* **11,** 431–437.

VERDOORN, T. A., KLECKNER, N. W., and DINGLEDINE, R. (1987). Rat brain NMDA receptors expressed in *Xenopus* oocytes. *Science* **238,** 1114–1116.

ZILLES, K., RATH, M., SCHLEICHER, A., GLASER, T., and TRABER, J. (1986). Ontogenesis of serotonin (5-HT) binding sites in the choroid plexus of the rat brain. *Brain Res.* **380,** 201–203.

ZILLES, K., SCHLEICHER, A., GLASER, T., TRABER, J., and RATH, M. (1985). The ontogenetic development of serotonin (5-HT_1) receptors in various cortical regions of the rat brain. *Anat. Embryol.* **172,** 255–264.