

# Partial purification and functional expression of brain mRNAs coding for neurotransmitter receptors and voltage-operated channels

(brain mRNA/*Xenopus* oocytes/membrane channels/transmitter receptors)

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**ABSTRACT** Poly(A)<sup>+</sup> mRNAs extracted from embryonic chicken brain and from adult rat brain were fractionated on sucrose density gradients. The fractions were subsequently injected into *Xenopus* oocytes where the mRNA was translated. The products were processed and incorporated into the oocyte membrane where they formed functional neurotransmitter receptors and voltage-operated channels. Different mRNA fractions induced the incorporation of different transmitter receptors and voltage-operated channels into the oocyte membrane. These experiments provide a useful step towards the understanding of the structure and function of neurotransmitter receptors and channels.

We have shown previously that poly(A)<sup>+</sup> mRNA, isolated from the brain of several animal species and injected into *Xenopus* oocytes, leads to the synthesis and incorporation of functional membrane channels activated by voltage or by neurotransmitter drugs (1-7). For example, injection of mRNA derived from chicken brain induced the oocytes to acquire voltage-activated sodium and potassium channels and also channels opened by  $\gamma$ -aminobutyric acid (GABA), kainate, and glutamate (1), whereas mRNA from adult rat brain induced these channels as well as voltage-activated calcium channels (6, 8) and channels opened by serotonin, GABA, glycine, and acetylcholine (AcCho) (2, 4). Those experiments were made with whole poly(A)<sup>+</sup> mRNA preparations, which clearly contained many different species of messengers coding for the different receptors and channels. In the present paper, we show that subfractions of the poly(A)<sup>+</sup> mRNA from rat and chicken brains, separated by sedimentation on sucrose density gradients, lead to the expression of specific receptors and voltage-activated channels in *Xenopus* oocytes.

## MATERIALS AND METHODS

Whole RNA (absorbance ratio  $A_{260}/A_{280}$  of 2.0) was extracted from optic lobes of 20-day-old chicken embryos and brains of adult Wistar rats by using techniques as described (9). Poly(A)<sup>+</sup> mRNA was then isolated by using oligo(dT)-cellulose and subsequently fractionated by sucrose density gradient centrifugation (10).

Methods for injection of mRNA into oocytes of *Xenopus laevis* and for electrophysiological recording were as used previously (9, 11-13). Data presented derive from oocytes of one donor injected with mRNA from rat brain and from two other donors injected with mRNA from chick brain. Electrophysiological recordings were made 5-10 days after injection of mRNA in oocytes treated with collagenase to

remove follicular and other enveloping cells (8).

Amplitudes of voltage-activated membrane currents were measured by holding the membrane potential at -100 mV and stepping the potential to less negative values. Sodium currents were measured by depolarizing the membrane to -10 mV; the transient outward chloride current ( $T_{out}$ ; see ref. 13), by depolarizing to 0 mV; and the fast potassium current, to +40 mV. Drug-induced currents were recorded with the potential clamped at -60 mV. In the case of the oscillatory currents elicited by glutamate, serotonin, and AcCho, the amplitude was taken as the peak height of the largest oscillation. Temperature was 18-22°C in all experiments.

## RESULTS

**Fractionation of mRNA.** Fig. 1 shows sedimentation profiles of mRNA fractions derived from chicken and rat brains. To study the functional expression of these fractions, we injected each oocyte with 50 nl of alternative fractions; because individual fractions contained different amounts of RNA (mRNA plus ribosomal RNA), the oocytes received between about 8 and 60 ng of RNA in the case of the chicken brain and between about 19 and 34 ng for rat brain. Several days after injection, individual oocytes were first examined for the presence of voltage-operated channels and then were exposed sequentially to various drugs to study the incorporation of transmitter receptors.

**Voltage-Activated Sodium and Potassium Currents.** After oocytes with whole poly(A)<sup>+</sup> mRNA were injected, the currents elicited by depolarizing pulses showed two main components that were not observed in control (noninjected) oocytes. These comprised an initial fast inward current, carried by sodium ions, and a slower outward current due to potassium ions (cf. ref. 3). Both currents began to appear at similar potentials, making it difficult to study each in isolation. However, after injecting oocytes with fractions of mRNA, we found that certain fractions induced the appearance of the inward sodium current alone, while others induced only the potassium current. This is illustrated in Fig. 2, which shows records from two oocytes injected with different fractions of chicken brain mRNA. Fraction 7 induced an inward sodium current, which became detectable with depolarization to about -40 mV and increased in size with further depolarization to -10 mV. As the potential was made more positive, the current declined and was almost completely suppressed at +40 mV (Fig. 2A). At this potential there was no activation of the potassium current, even

Abbreviations: AcCho, acetylcholine; GABA,  $\gamma$ -aminobutyric acid;  $T_{out}$ , transient outward current.

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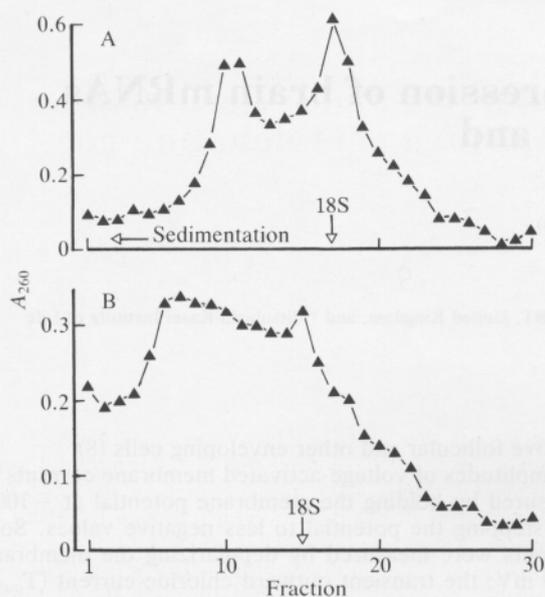


FIG. 1. Sedimentation profiles of poly(A)<sup>+</sup> mRNA derived from chicken optic lobe (A) and rat brain (B). After centrifugation, fractions (ca. 0.4 ml) were collected, precipitated with ethanol, washed with 70% ethanol, dried, and finally dissolved in 10  $\mu$ l of water. The arrows indicate the position of the 18S rRNA sedimented in parallel sucrose gradients.

though this continued to increase as the potential was made more positive (Fig. 2B; see also ref. 3). In complete contrast, oocytes injected with mRNA fraction 11 showed outward potassium currents on depolarization (Fig. 2B) but no inward current.

Profiles indicating the extent to which different mRNA fractions induced the appearance of functional sodium and potassium channels are shown in Fig. 3 (mRNA from chicken brain) and Fig. 4 (rat brain mRNA). The mRNAs coding for sodium channels sedimented predominantly in one or two fractions, which were at the same positions in the gradients (relative to the peak of the 18S rRNA) in both rat and chicken brain preparations. The messengers coding for potassium channels peaked 2–4 fractions lighter in the gradients. With both chicken and rat mRNA, the fractions that gave maximal sodium currents induced almost no potassium current, and vice versa.

The whole-rat-brain mRNA, from which the fractions were derived, was injected into oocytes from the same donor as was used to test the fractions. This particular donor was less effective than most at expressing the exogenous mRNA, and the whole mRNA induced only small sodium and potassium currents, which were less than 10% of the peak values obtained with the fractionated mRNA (Fig. 4).

**T<sub>out</sub>.** Depolarization of native *Xenopus* oocytes to around 0 mV elicits a T<sub>out</sub> current, which is carried by chloride ions and depends upon a voltage-activated influx of calcium (8, 13, 14). This current is enhanced in oocytes injected with whole mRNA from rat brain (6) probably because the messenger induces the formation of additional calcium channels in the membrane (8). Fig. 4C shows the size of the T<sub>out</sub> current in oocytes injected with different fractions of rat brain mRNA. The current in oocytes injected with most fractions was not much greater than the native response in noninjected oocytes. However, fraction 13 mRNA clearly induced a larger T<sub>out</sub> current.

**Drug-Activated Currents.** To assess the extent to which different fractions of mRNA caused the expression of drug-activated membrane channels, we recorded currents elicited by bath perfusion of drugs. Fig. 5 shows responses to

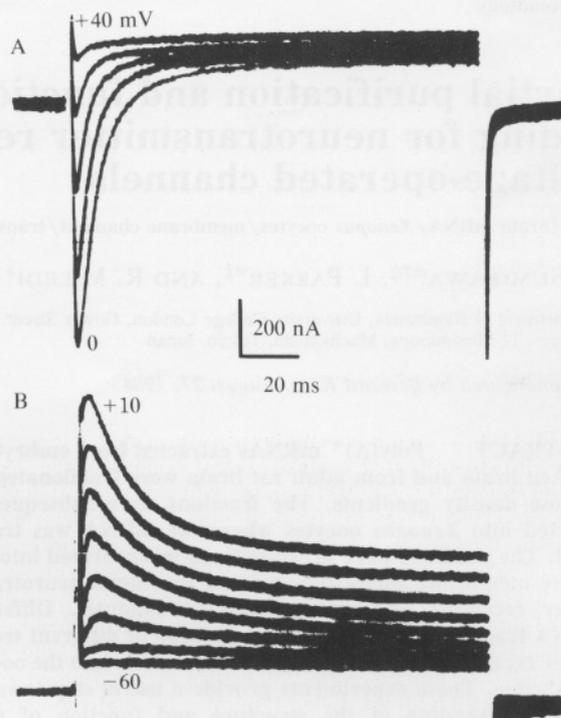


FIG. 2. Voltage-activated sodium (A) and potassium (B) currents recorded from two oocytes injected with different fractions of mRNA from chicken optic lobe. Downward deflexions in this and other records correspond to inward membrane currents. (A) Records from an oocyte injected with fraction 7 mRNA. The membrane potential was held at  $-100$  mV and stepped to potentials of (from bottom to top) 0, +10, +20, +30, and +40 mV. (B) Records from an oocyte from the same donor injected with mRNA fraction 11. The potential was held at  $-100$  mV and stepped to (from bottom to top)  $-60$ ,  $-50$ ,  $-40$ ,  $-30$ ,  $-20$ ,  $-10$ , 0, and +10 mV.

kainate, glycine, and GABA in two oocytes injected with fractions 11 and 13 from rat brain. At a clamp potential of  $-60$  mV, all of these drugs elicited smooth inward membrane currents (4, 5), but the sizes of the responses were strikingly different. Both fractions induced receptors to kainate, but while oocytes injected with fraction 13 gave large responses to glycine and none to GABA, fraction 11 induced larger response to GABA than to glycine.

Profiles indicating the extent to which the messenger fractions induced sensitivity to different drugs are shown in Fig. 3 A and B (chicken brain) and Fig. 6 (rat brain). Just as with the voltage-operated channels, the messengers coding for GABA and kainate receptors in both chicken and rat brains sedimented at similar positions (relative to the 18S rRNA). Induction of some receptors (e.g., kainate and muscarinic AcCho) was widely spread among different fractions, while others (e.g., GABA) were more sharply peaked. In the rat brain mRNA preparation, there was a gradual increase in expression of some receptors by denser fractions distinct from the main peak of activity (Fig. 6). This may have resulted from aggregation of mRNAs coding for these receptors, causing them to sediment further down the gradient.

As was the case for the voltage-operated channels, the drug responses induced by injection of whole poly(A)<sup>+</sup> mRNA from rat brain were appreciably smaller than the responses obtained after injection of mRNA fractions (Fig. 6).

**Fractionation of Glutamate Responses.** Application of glutamate to oocytes injected with whole mRNA from rat brain elicits two types of membrane currents (4). One is a slow, oscillatory chloride current, which inverts direction at

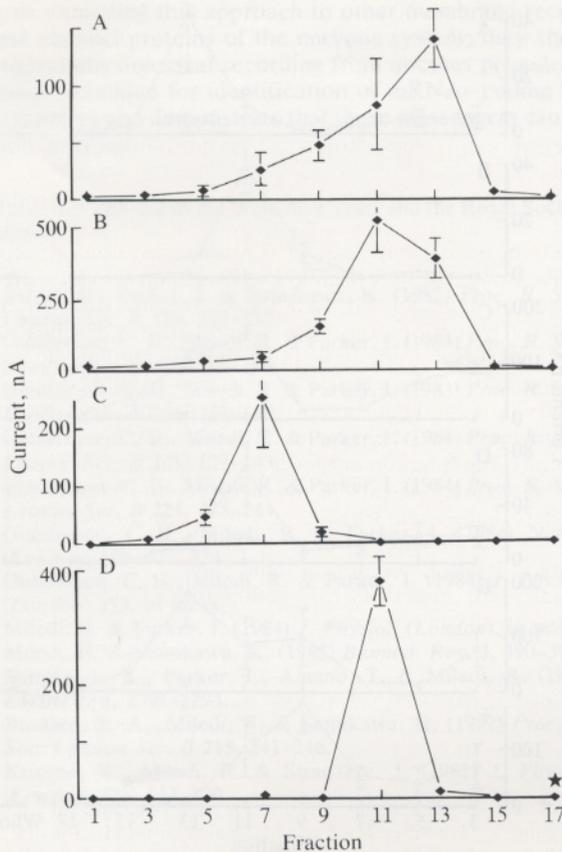


FIG. 3. Profiles of drug (A and B)- and voltage (C and D)-activated responses induced by mRNA fractions derived from chick brain. (A) GABA. (B) Kainate. (C) Sodium. (D) Potassium. Each point is a mean value obtained from measurements on 4–11 oocytes. Error bars indicate  $\pm 1$  SEM and are smaller than the symbol height where not shown. Measurements of drug-activated currents (A and B) were obtained from one donor and of voltage-activated currents (C and D), from a different donor. GABA was applied at a concentration of 1 mM, and kainate at 0.1 mM. The star marks the fraction in which the 18S rRNA sedimented.

about  $-30$  mV. The other is a smooth current, which inverts at about 0 mV. These two components superimpose and are usually difficult to discriminate, although the longer latency of the oscillatory chloride current sometimes allows the faster smooth current to be distinguished (cf. ref. 4).

Different fractions of mRNA from rat brain induced these two components to different extents. For example, Fig. 7 shows an oscillatory response to glutamate in an oocyte injected with fraction 11 mRNA, and a smaller, smooth response in an oocyte injected with fraction 13. The smooth response inverted at about 0 mV, while the oscillatory response in oocytes injected with fraction 11 inverted at more negative potentials. The receptors mediating the smooth response to glutamate were probably induced also by fraction 11 mRNA, since responses often showed an initial steady rise before the first oscillatory spike (Fig. 7A). However, it was difficult to estimate separately the sizes of each component, and the profile of glutamate sensitivity in Fig. 6 simply shows the total amplitude of the current elicited by glutamate.

## DISCUSSION

We have shown previously that whole mRNA from brain and muscle cells induces the *Xenopus* oocyte membrane to acquire receptors and channels which function as those in the original cell membranes (1–5, 11, 15, 16). The present

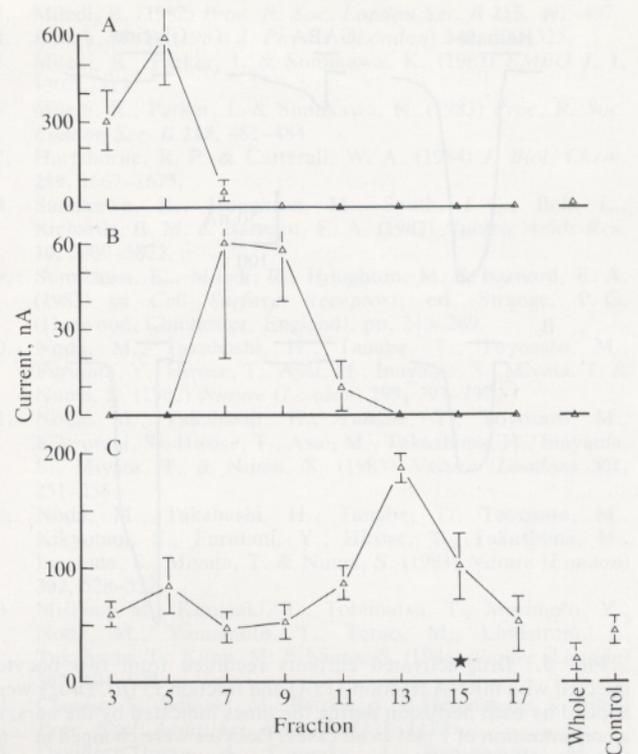


FIG. 4. Profiles of voltage-activated responses induced in oocytes injected with fractions of mRNA derived from rat brain. (A) Sodium. (B) Potassium. (C)  $T_{out}$ . Each point is a mean value from 7–14 oocytes. Points on the right indicate mean currents from oocytes that were injected with about 70 ng of whole poly(A)<sup>+</sup> mRNA. The  $T_{out}$  current in control (noninjected) oocytes is also shown. All oocytes were obtained from one donor. The star marks the position of the 18S rRNA.

experiments show that fractionated mRNA from rat and chicken brains is still effective in expressing many voltage- and drug-operated membrane channels. Moreover, induction of particular channels tended to be restricted to certain fractions that had similar sedimentation properties in both chicken and rat brain mRNAs (cf. Figs. 3, 4, and 6). This suggests that the mRNAs and, hence, the structures of the translated proteins are similar in these two species.

The use of fractionated rather than whole poly(A)<sup>+</sup> mRNA offers important advantages for the study of membrane channels "transplanted" in this way into the oocyte. For instance, specific messengers are more concentrated, so that larger responses are obtained. More importantly, by injecting the appropriate fraction, it becomes possible to incorporate only the desired channel type. For example, whole mRNA from the brain induces voltage-gated sodium and potassium channels; but, like in the native cells, it is difficult to study each channel in isolation without using pharmacological blocking agents. However, sodium channels can be studied without interference from potassium channels in oocytes injected with fraction 5 of rat brain mRNA, while fraction 9 expresses the potassium but not the sodium channels (cf. Fig. 4).

The interpretation of the "sedimentation profiles" of the various receptors and channels (Figs. 3, 4, and 6) is at present unclear. If each receptor/channel were coded by a single mRNA species or by several messengers of similar size, then sharp peaks would be expected. On the other hand, if the receptor/channels were made of several protein subunits, all of which were required to form a functional complex, and if the individual messengers were of very different sizes sedimenting in different fractions, then no

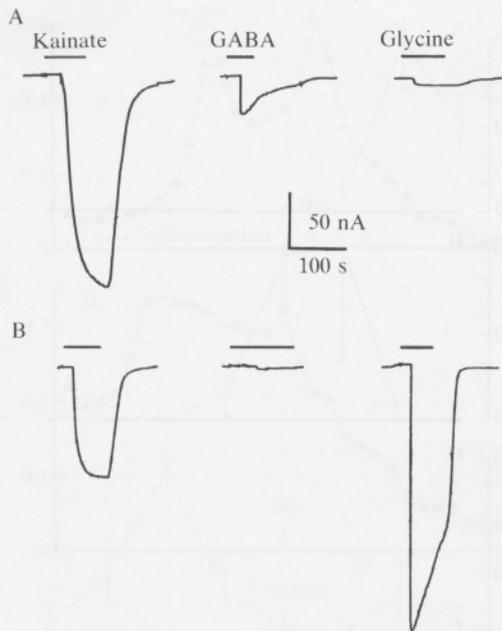


FIG. 5. Drug-activated currents recorded from two oocytes injected with mRNA fraction 11 (A) and fraction 13 (B). Drugs were applied by bath perfusion during the times indicated by the bars, at a concentration of 1 mM in all cases. Oocytes were clamped at  $-60$  mV.

fraction would be able to express the channels. This is clearly not what was found, because fractionated mRNA was even more effective than whole mRNA in inducing the receptors and channels examined. Moreover, the sedimentation profiles were a mixture of peaks (e.g., sodium and potassium) and widespread distributions (e.g., muscarinic AcCho). Such a pattern may arise if some functional channels are coded by a single mRNA species, while in others the same proteins are coded by messengers of different sizes (cf. ref. 10) or some messengers aggregate.

For example, the sodium channel purified from rat brain consists of three subunits,  $\alpha$ ,  $\beta_1$ , and  $\beta_2$ , with molecular weights of 260,000, 39,000, and 37,000 (17). Our results show that the mRNA coding for the sodium channel sedimented near the bottom of the gradient, as might be expected for the mRNA coding for the heavy subunit, but perhaps not for mRNAs encoding the smaller subunits. Thus, it could be that only the large subunit is required to form a functional sodium channel. Alternatively, if two or three subunits are required, then it seems that these are coded by mRNAs of similar sizes, despite the large difference in molecular weights of the translation products, or the messengers coding for the small subunits aggregate. The picture may become clearer when other methods are used to separate specific messengers.

Fraction 13 of the rat brain mRNA gave responses to glycine but not GABA, whereas fraction 11 gave larger responses to GABA than to glycine (Fig. 5). Thus, the mRNAs coding for these receptors sedimented separately, even though both receptors operate by opening chloride-selective membrane channels (1, 2, 4). Thus, it seems very likely that GABA and glycine receptors are coded by different mRNAs. However, we still do not know if the associated chloride channels are the same and were induced by the foreign mRNA, or whether the channels are those already present in the native oocyte membrane. In oocytes injected with fraction 11 mRNA, the responses to GABA were enhanced by barbiturates and benzodiazepines (unpublished data). This suggests that the binding sites for these modulating drugs are coded by the same or similar-sized mRNA to that which codes for the GABA binding site.

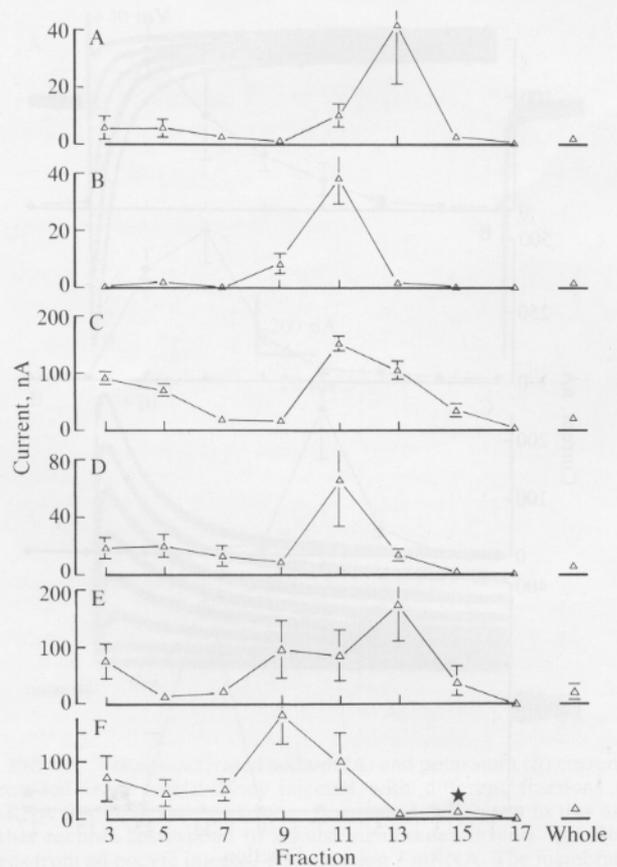


FIG. 6. Profiles of drug-activated currents induced by fractions of mRNA obtained from rat brain. (A) Glycine, 1 mM. (B) GABA, 1 mM. (C) Kainate, 0.1 mM. (D) Glutamate, 0.01 mM. (E) AcCho, 0.1 mM. (F) Serotonin, 0.1  $\mu$ M. Each point is a mean value from 4–11 oocytes. Values on the right were obtained from oocytes injected with the whole poly(A)<sup>+</sup> mRNA that was used to derive the fractions. Values of the glutamate responses (D) give the peak current, including both oscillatory and smooth components. The responses to AcCho (E) were oscillatory currents due to activation of muscarinic and not nicotinic receptors (cf. ref. 4). The star marks the position of the 18S rRNA.

The partially purified mRNAs that we examined may prove useful in cloning the genes coding for brain receptors and channels. Once such genes are identified, their nucleotide sequences can be determined, hence allowing prediction of the encoded amino acid sequences in the protein. Furthermore, the cloned genes can be expressed in the oocyte system, allowing the structure–function relationship to be studied. This approach has been successfully used for the nicotinic AcCho receptor (18–25). Our results are a step

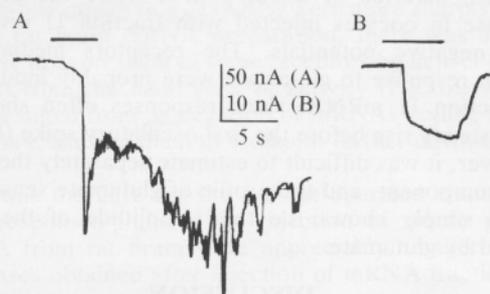


FIG. 7. Oscillatory and smooth currents elicited by glutamate in oocytes injected with fractions 11 (A) and 13 (B) from rat brains. Clamp potential was  $-60$  mV in both cases, and glutamate (0.01 mM) was applied as indicated by the bars.

towards extending this approach to other membrane receptor and channel proteins of the nervous system; they show that electrophysiological recording from oocytes provides a powerful technique for identification of mRNAs coding for these proteins and demonstrate that these messengers can be partially purified.

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