Another effect of GABA on motor axons is the faster repolarization of the action potential. Since this effect persisted in the presence of the K^+ channel blocker Cs^+, it may be suggested that GABA was not modulating a voltage-gated K^+ conductance. In cultured dorsal root ganglion cells GABA reduces action potential duration by a selective decrease in a voltage sensitive Ca^{2+} conductance[15]. Several lines of evidence suggest that a similar mechanism is not readily applicable to our data, since, for example, in squid axons the late Ca^{2+} current cannot be detected under voltage clamp[16]. In any event calcium spikes disappear from *Xenopus* neurites during development[17] and transmitter release at the frog neuromuscular junction, a process exquisitely sensitive to calcium, is not modified by GABA[18]. One possible explanation for our data is that the decreased input resistance caused by GABA, presumably mediated by chloride ions like most other GABA responses[2], reduced the membrane time constant and consequently shortened the spike. Clearly this hypothesis requires further investigation.

The physiological significance of GABA receptive sites on motor axons is unclear because the synaptic input does not extend beyond the axon hillock[19] and we used rather a high concentration of this amino acid to elicit axonal responses. It might well be that GABA-sensitive sites are widely present on nerve membranes during development and that they have only a vestigial role in the adult animal. Perhaps more important are the pharmacological implications of our results since attempts to treat neurological conditions characterized by a deficit of GABA (e.g. Huntington's chorea) with systemic administration of GABA agonists[20] may affect extrasynaptic sites (for instance on axons) and contribute to side effects such as muscle hypotonia[21].

1 The authors gratefully acknowledge support from The Joint Research Board of St. Bartholomew's Hospital.
2 Nistri, A., and Constanti, A., Progr. Neurobiol. *13* (1979) 117.
3 Dunlap, K., Br. J. Pharmac. *74* (1981) 579.
4 Desarmenien, M., Feltz, P., Loeffler, J.P., Occhipinti, G., and Santangelo, F., Br. J. Pharmac. *76* (1982) 289P.
5 Krnjević, K., Puil, E., and Werman, R., Can. J. Physiol. Pharmac. *55* (1977) 658.
6 Brown, D.A., and Marsh, S., Brain Res. *156* (1978) 187.
7 Schwindt, P.C., in: Frog neurobiology, p.750. Eds R. Llinás and W. Precht. Springer, Berlin 1976.
8 Arenson, M.S., and Nistri, A., J. Physiol., Lond. *319* (1981) 24P.
9 Davidoff, R.A., and Adair, R., J. Neurochem. *24* (1975) 545.
10 Allan, R.D., Evans, R.H., and Johnston, G.A.R., Br. J. Pharmac. *70* (1980) 609.
11 Barrett, E.F., and Barrett, J.N., J. Physiol., Lond. *323* (1982) 117.
12 Puil, E., and Werman, R., Can. J. Physiol. Pharmac. *59* (1981) 1280.
13 Cahalan, M., in: Physiology and pathobiology of axons, p.158. Ed. S.G. Waxman. Raven Press, New York 1978.
14 Matthews, G., and Wickelgren, W.O., J. Physiol., Lond. *293* (1979) 393.
15 Dunlap, K., and Fischbach, G.D., Nature *276* (1978) 837.
16 Hagiwara, S., and Byerly, L., A. Rev. Neurosci. *4* (1981) 69.
17 Willard, A.L., J. Physiol., Lond. *301* (1980) 115.
18 Smart, T.G., Br. J. Pharmac. *71* (1981) 279.
19 Conradi, S., in: The peripheral nerve, p.295. Ed. D.N. Landon. Chapman and Hall, London 1976.
20 Shoulson, I., Chase, T.N., Roberts, E., and Van Balgooy, J., N. Engl. J. Med. *293* (1975) 504.
21 Barbeau, A., Lancet *2* (1973) 1499.

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Experimental catalepsy: Influences of cholinergic transmission in restraint-induced catalepsy

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Summary. Possible cholinergic mechanisms in experimental catalepsy were evaluated by using the 'pinch-induced' model in mice. In control, saline-injected mice, the median number of attempts needed to achieve a criterion level of catalepsy was 6. All 3 dose levels of physostigmine reduced this median to about 2 trials; neostigmine did not significantly reduce the number of trials. Opposite effects were obtained with atropine, with which all 3 doses tested increased the number of trials needed to cause catalepsy, and at the higher doses (5 and 10 mg/kg) most of the mice (80%) became insusceptible; atropine methyl bromide had no such effects. Thus, this kind of catalepsy may be mediated by cholinergic mechanisms that are central and not peripheral.

Catalepsy, which is commonly defined[1] as an immobile condition of waxy flexibility where the subject tends to remain in any imposed position, has been studied experimentally in various ways. One method which does not involve the use of drugs, such as neuroleptics or opiates[2,3], is a restraint-induced catalepsy (RIC). A technique for inducing RIC in mice, which is probably the same or akin to the reflex immobility state popularly known as 'animal hypnosis'[4,5], has recently been described by Amir et al.[6].

RIC is especially useful for studying cataleptic mechanisms because it can be produced without the confounding influence of drugs; one can therefore evaluate the effect of drugs which have known actions on specific neurotransmitter systems. This report describes the effects of drugs which alter function of the transmitter acetylcholine. The results strongly indicate that RIC is a central nervous system phenomenon that is dependent upon acetylcholine's action on muscarinic receptors.

RIC was produced and scored as previously described[6]: young adult outbred mice (25–30 g) were held on a flat surface by the scruff of the neck, using the thumb and index finger. After being kept immobile for 5 sec, the mouse was placed in an abnormal posture, with both forefeet placed on the top of a 5-cm wall. Initially, mice quickly removed their feet from the wall, but after about 6 such trials, with 20-sec inter-trial intervals, all of the undrugged mice would remain motionless with feet elevated for a criterion duration of 20 sec (fig. 1). The number of trials needed to reach criterion immobility, as well as the percentage of susceptible animals, provided the frame of reference for evaluating the cholinergic drugs.

Each test group contained 10 mice. Solutions were in concentrations that permitted the same volume to be injected irrespective of drug. The observor who scored the RIC did not know which drug, or saline, had been injected. Normal cholinergic function can be augmented by inhibit-

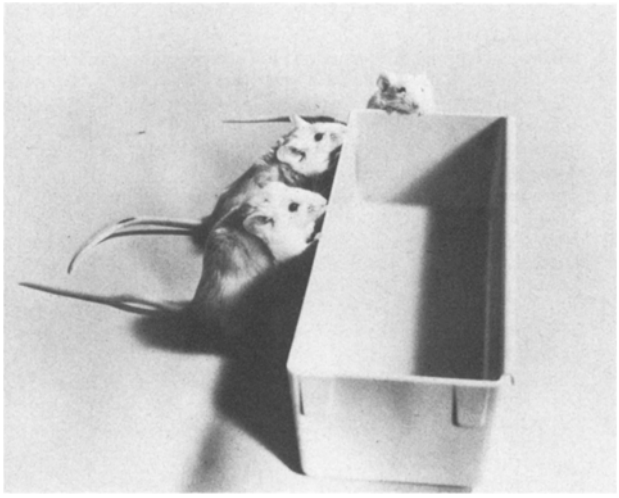

Figure 1. Illustration of the restraint-induced catalepsy in 3 mice which remained in this immobile position for 20 or more sec, which was the criterion used for a cataleptic response.

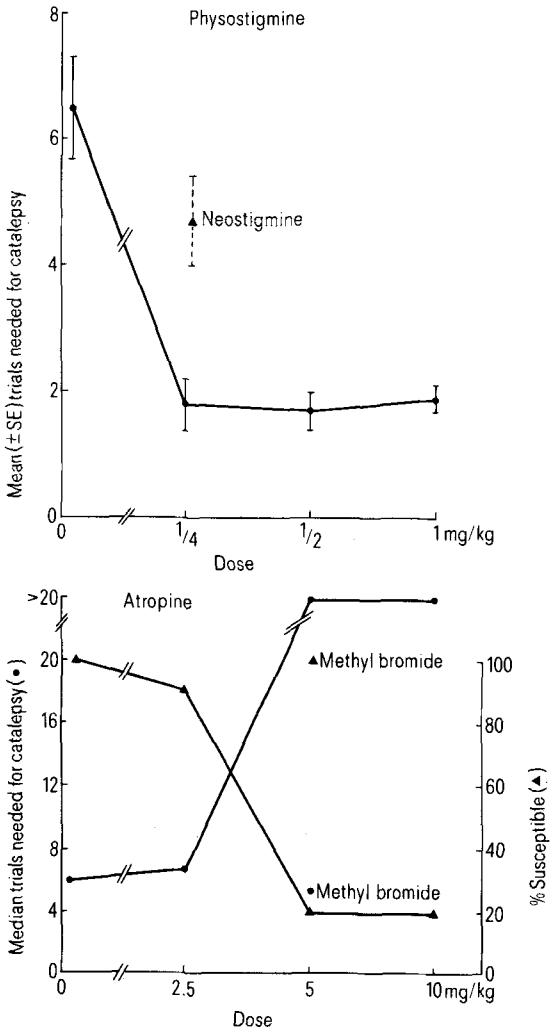

Figure 2. Enhancement of restraint-induced catalepsy by physostigmine (but not by neostigmine) (above) and the interference with the catalepsy by atropine sulfate (but not by atropine methylbromide) (below).

ing the enzyme, acetylcholinesterase, which normally destroys endogenous acetylcholine. When mice were injected i.p. 15 min prior to RIC trials with the cholinesterase inhibitor physostigmine, there was a clear enhancement of the state (fig. 2). Compared with the saline-injected control mice, which had a mean of 6 trials to reach criterion, all doses of physostigmine resulted in a mean number of trials less than 2. The catalepsy persisted even though at the high doses there was a conflicting behavior of violent trembling and shaking. As a test of whether the physostigmine effect was central or peripheral, another group of mice was tested with neostigmine, a cholinesterase inhibitor which acts peripherally, but which does not cross the blood-brain-barrier well. The lowest dose (higher doses were lethal, as expected[7]) produced results similar to controls, suggesting that the important consequence of acetylcholine buildup is in the brain and spinal cord.

When cholinergic receptors were blocked by 15-min pretreatment with an i.p. injection of atropine sulfate, mice became difficult to immobilize. A dose-dependent interference with RIC was manifest in greater number of trials needed to reach criterion (left vertical axis of fig. 2) and in a lower percentage of susceptibility (right axis of fig. 2). At higher atropine doses 80% of the mice were completely insusceptible, even though they were behaviorally very sedated. Paradoxically, they quickly removed their feet from the ledge, even though they were quite hypokinetic and apparently sleepy immediately before and after each RIC trial.

As a test for central vs peripheral action, another group of mice was tested with atropine methyl bromide, which blocks muscarinic synapses peripherally but which does not cross the blood-brain-barrier well. These mice responded similarly to controls, with a low number of trials needed to reach criterion and with 100% susceptibility.

The atropine-induced disruption of RIC obviously suggests a muscarinic mechanism. However, in another group of 20 mice, a moderate dose (4 mg/kg) of the nicotinic blocker, mecamylamine, had atropine-like effects (median number of trials to criterion was increased over 20 and the percentage susceptible was reduced to 42%).

Thus, the possibility is raised that the physostigmine enhancement of RIC is mediated via both muscarinic and nicotinic receptors. However, the results could be explained another way, namely that RIC and cholinomimetics produce separate behavioral states that are compatible and additive. Physostigmine promotes a behavioral state of reduced locomotion, which would complement RIC. The cholinergic blockers may produce locomotor disinhibition or have similar effects that would be incompatible with the immobility of RIC.

These results are consistent with the earlier postulate[8] that cholinergic systems mediate forms of behavioral inhibition such as 'freezing'; a cholinergic basis for decreased avoidance and escape behavior has been recently reported[9]. Another laboratory[10] has reported that cholinergic mechanisms were clearly implicated in a behavioral test where mice were put on a string-wrapped vertical rod. Either muscarinic or nicotinic agonists reduced movement on the rod, and the corresponding antagonists interfered with that effect.

Drug effects similar to what I obtained with RIC have been reported in studies of 'animal hypnosis' in chickens and rabbits[11,12], but the results are much too inconsistent[13-15] to permit a definite conclusion that hypnosis and RIC have a similar cholinergic basis. The present results point out the need to evaluate neuroleptic effects on RIC; in rats, either lesions of dopaminergic pathways or atropine reportedly interfered with the catalepsy that was induced by haloperidol[2]. Such results have led to theories of catalepsy that are

based on an interaction of cholinergic and dopaminergic systems, particularly in the striatum[2]. Therefore, there is a need to see if neuroleptics enhance RIC and if atropine or mecamylamine diminish that effect while physostigmine enhances it.

1 Dorland, W.A.N., The American illustrated medical dictionary, 22nd edn. W.B. Saunders, Philadelphia 1951.
2 Costall, B., and Naylor, R.J., Psychopharmacologia *34* (1974) 233.
3 Ezrin-Waters, C., Muller, P., and Seeman, P., Can. J. Physiol. Pharmac. *54* (1976) 516.
4 Klemm, W.R., in: Neurosciences research, vol.4, p.165. Eds S. Ehrenpreis and O.C. Solnitzky. Academic Press, New York 1971.
5 Klemm, W.R., J. Neurosci. Res. *2* (1976) 57.
6 Amir, S., Brown, Z.W., Amit, Z., and Ornstein, K., Life Sci. *28* (1981) 1189.
7 Barnes, C.D., and Eltherington, L.G., Drug dosage in laboratory animals. Univ. California Press, Berkeley 1964.
8 Carlton, P.L., in: Reinforcement and behavior, p.286. Ed. J.T. Tapp. Academic Press, New York 1969.
9 Anisman, H., Psychopharmacology *74* (1981) 81.
10 Zetler, G., Neuropharmacology *10* (1971) 289.
11 Thompson, R.W., Psych. Rec. *1* (1977) 109.
12 Thompson, R.W., Physiol. Psychol. *7* (1979) 454.
13 Ksir, C., Physiol. Psychol. *6* (1978) 521.
14 Hatton, D.C., Woodruff, M.L., and Meyer, M.E., J. comp. Physiol. Psychol. *89* (1975) 1053.
15 Woodruff, M.L., Hatton, D.C., Frankl, M.B., and Meyer, M.E., Physiol. Psychol. *4* (1976) 198.

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Fast decay of fish synaptic currents[1]

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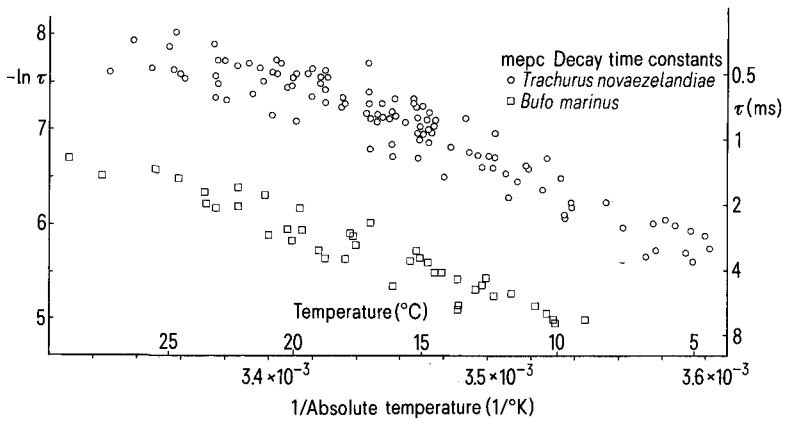
Summary. Teleost miniature end plate currents decay more rapidly than those of birds or tetrapods, with an exponential time constant of about 1 msec at 15 °C, but are otherwise normal. The rapid decay is correlated with increased unsaturation and greater fluidity of fish lipids.

The correlation between anesthetic potency and lipid solubility, which the synergistic/antagonistic effects of temperature/pressure on anesthesia, have led to hypotheses that all 3 agents interact with membrane lipids to alter fluidity, either in bulk, or in localized domains adjacent to ion channels[2,3]. The vertebrate neuromuscular junction has furnished support for this hypothesis[4], with the duration of miniature end plate currents (MEPCs) being decreased by anesthetics[5,6], higher alcohols[7,8], and elevated temperature[9]. A likely mode of action for these agents is an increase in membrane fluidity in the immediate vicinity of transmembrane channels, permitting faster relaxation of molecular gates.

Lipids extracted from the brains of fishes are more unsaturated (hence more fluid) than those of terrestrial vertebrates[10], and the fluidity of fish synaptic membranes has been correlated with lipid unsaturation[11]. Thus a comparative study of fish postsynaptic currents should serve as a test of the lipid fluidity hypotheses, which would predict that end plate currents in the more fluid fish membrane would decay more rapidly than those in tetrapods at any given temperature.

Specimens of *Trachurus novaezelandiae* Richardson, 1843 (Teleostei: Carangidae)[12] were captured in Port Jackson, Sydney, Australia, at an ambient temperature of 23 °C, and kept at 12–15 °C in a recirculating aquarium for 1 to 15 days. The inferior oblique extraocular muscle was dissected free and pinned in a perfusion chamber filled with physiological saline (231 mM NaCl, 2.7 mM KCl, 2.25 mM $CaCl_2$, 3.67 mM $MgCl_2$, 10 mM HEPES, pH 7.2). Focal extracellular recordings of spontaneous miniature endplate currents (MEPCs) were made from the white fiber band of the muscle with firepolished, blunt (30 µm) electrodes filled with 1 M NaCl or physiological saline. Because teleost motor innervation is usually distributed along the length of the muscle fiber, rather than being localized as in amphibia and mammals[13], end plates could not be located visually, but were found by sweeping the muscle surface with the



Comparison of MEPC decay time constants (τ) from fish (*Trachurus novaezelandiae*: circles) and toad (*Bufo marinus*: squares) muscle; Arrhenius plot. Each point represents the mean of 3–320 individual MEPCs, recorded by the same experimentor using identical techniques. Time constants for both animals are inverse exponential functions of temperature, but the fish MEPCs are consistentyl 3–4 times faster than toad MEPCs.