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THE EFFECT OF REPETITIVE STIMULATION UPON MONOSYNAPTIC TRANSMISSION IN KITTENS

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A number of differences have been described in the functional properties of the monosynaptic reflex pathway in the lumbosacral spinal cords of kittens and of adult cats (Malcolm, 1953, 1955; Skoglund, 1960*a*, *b*; Wilson, 1962; Eccles, Shealy & Willis, 1963; Eccles & Willis, 1963). For example, facilitation of monosynaptic reflexes was observed to develop in kittens over 6 days old (Malcolm, 1955). Skoglund (1960*a*) confirmed that young kittens differed from adult cats in that monosynaptic reflexes were not facilitated by homonymous afferent volleys that themselves were maximal for producing monosynaptic reflex discharges. Sometimes there was a relative facilitation lasting up to 10 msec (Skoglund, 1960*a*) but often there was no response at brief test intervals. Furthermore, no post-tetanic potentiation of the monosynaptic reflex could be demonstrated in young kittens (Skoglund, 1960*b*; Wilson, 1962), in contrast to the findings in adult cats (e.g. Lloyd, 1949).

The present work was undertaken to investigate further the effects of repetitive stimulation upon monosynaptic transmission in kittens. The evidence so far obtained suggests at least a partial explanation for some of the differences between the behaviour of the monosynaptic pathway in kittens and in adult cats.

METHODS

Kittens ranging in age from 10 to 41 days were anaesthetized with pentobarbital sodium and spinalized at L1. The spinal cord and several peripheral nerves of the hind limb were prepared as described previously (Eccles, Shealy & Willis, 1963). In very young kittens (under 14 days), no attempt was made to record intracellularly from motoneurones; monosynaptic transmission was tested by recording reflex discharges from the cut central ends of the appropriate ventral roots or, in a few experiments, from peripheral nerves. In older animals, intracellular recording of monosynaptic excitatory post-synaptic potentials (EPSPs) was employed as well, using standard techniques (Brock, Coombs & Eccles, 1952). The micro-electrodes were filled with 3 M-KCl and initially had resistances of 5–10 MΩ.

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Because of the complication of presynaptic inhibition and the associated large dorsal root reflexes which would be expected from the use of afferent volleys in flexor nerves (Eccles & Willis, 1963), afferent volleys in extensor nerves were employed except for one case (Fig. 5*C* and *D*). The names of the extensor nerves and their abbreviations are as follows: medial gastrocnemius (MG), lateral gastrocnemius and soleus (LGS), both gastrocnemius nerves plus soleus (GS), semimembranosus and anterior biceps (SMAB), plantaris (PL), and the combined flexor longus digitorum and flexor hallucis digitorum nerves (FDHL). The only flexor nerve employed was posterior biceps-semitendinosus (PBST). Unless otherwise stated all records illustrated consist of several superimposed traces.

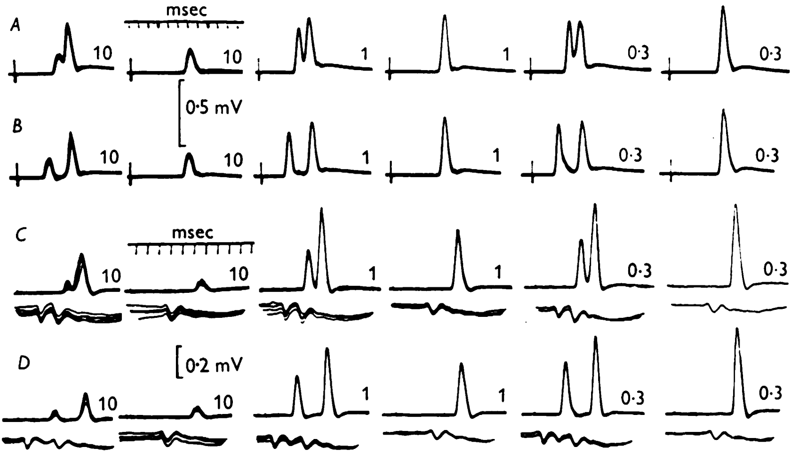

Fig. 1. The monosynaptic reflex from LGS is recorded in L7 ventral root in the second, fourth and sixth columns at rates of 10/sec, 1/sec and 0·3/sec respectively. In the first, third and fifth columns these reflexes were conditioned by a volley in MG which itself evoked a reflex at the same repetition rates, i.e. 10/sec, 1/sec and 0·3/sec. The upper two rows (*A* and *B*) were recorded in a 10-day-old kitten; potential scale 0·5 mV and time scale indicated above row *A*. The lower two rows (*C* and *D*) were from a 19-day-old kitten; potential scale 0·2 mV and time scale indicated above row *C*. The lower traces (in *C* and *D*) were recorded by an electrode making contact with the L7 dorsal root as it entered the spinal cord.

RESULTS

To explain the lack of facilitation of kitten monosynaptic reflexes by supramaximal conditioning volleys, Skoglund (1960*a*) suggested that the very long refractory period postulated for immature afferent terminals (cf. Hursh, 1939) might prevent invasion of the terminals by the test volley. Implicit in this proposal is a further assumption to explain the facilitation produced when the conditioning volley is subliminal for evoking reflex discharges. Skoglund presumed that the lowest threshold afferent fibres are more mature than the higher threshold ones, and so their terminals would support two successive impulses within the period required for demonstrating the facilitation. However, this explanation would not obtain for results such as those illustrated in Figs. 1 and 2.

Figure 1 shows records from two similar experiments, one (*A*, *B*) from a 10-day old kitten and the other (*C*, *D*) a 19-day-old kitten. The records are assembled in pairs. The left trace of each pair shows two monosynaptic reflex spikes, the right trace a single reflex spike. In *A* the double reflex spikes were evoked by stimulation first of the MG and then 1·43 msec later of the LGS nerve. The single spikes were produced by stimulation of the testing LGS nerve alone. The left-hand pair of records in Fig. 1*A* was evoked by stimuli repeated at a rate of 10/sec, the middle pair at 1/sec and the right-hand pair at 0·3/sec. In Fig. 1*B* the interval between the conditioning MG volley and the test LGS volley was increased to 2·76 msec and there were similar frequencies of repetition. Similarly, Fig. 1*C* and *D* show some of the records from an extensive series in a 19-day-old kitten. The conditioning-test intervals illustrated are 1·2 and 2·8 msec for Fig. 1*C* and *D*, respectively.

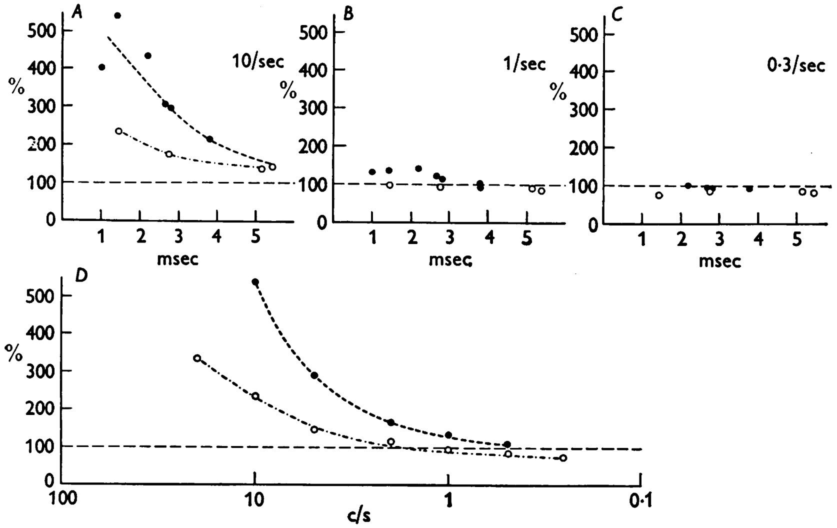

Fig. 2. The reflexes partly illustrated in Fig. 1 are plotted to show the differences in reflexes between a 10-day-old kitten (open circles) and a 19-day-old kitten (filled circles). The LGS reflexes facilitated by MG are expressed as a percentage of the height of the unfacilitated LGS reflex (ordinate); 100% being the control; the interval between the MG and LGS reflexes being the abscissa. In *A* the reflexes were recorded at a repetition rate of 10/sec; in *B* 1/sec and in *C* 0·3/sec. In *D* at a fixed interval of 1·43 msec for the 10-day-old kitten and 1·4 msec for the 19-day-old kitten the percentage is plotted against the frequency (abscissa).

Figure 2 summarizes graphically the results of these two experiments. The time courses of the changes in the LGS monosynaptic reflex produced by the action of the MG conditioning volley are shown in Fig. 2 *A*–*C* for the three repetition rates illustrated in Fig. 1. The amplitudes of the reflex spikes are plotted as a percentage of the control height for several conditioning-test intervals, including those of Fig. 1. There was no facilitation of the monosynaptic reflex of either kitten at any interval when the repetition rate was 0·3/sec. In the 10-day-old kitten (Fig. 2*C*, open circles), there was in fact a slight depression. On the contrary, when the repetition rate was 10/sec (Fig. 2*A*), there was a pronounced facilitation in each of the experiments. At a rate of 1/sec (Fig. 2*B*), there was only a slight facilitation with the 19-day-old kitten.

The graph of Fig. 2*D* shows the effect of varying the repetition rate over a wide range of frequencies upon the amount of facilitation; the conditioning-test intervals in Fig. 2*D* were constant at 1·43 msec for the 10-day-old kitten (open circles) and 1·4 msec for the 19-day-old kitten (filled circles).

The absence of facilitation between heteronymous synergic volleys (Fig. 2*B*, *C*) cannot be attributed to the long refractory period of the presynaptic terminals of the afferent fibres (cf. Skoglund, 1960*a*). Inhibition, either Group Ib (Laporte & Lloyd, 1952; Eccles, Eccles & Lundberg, 1957*b*) or recurrent (Renshaw, 1946; Eccles, Fatt & Koketsu, 1954; Skoglund, 1960*a*), could be invoked to account for an apparent lack of facilitation. However, the curves of Fig. 2*A*–*C* give no indication that inhibitory phenomena play any important role. It is, therefore, necessary to consider other explanations for the absence of facilitation which may be observed in kittens. One possibility is that there might not be an adequate subliminal fringe upon which to display a facilitation.

The proportion of the motoneuronal pool discharged by single afferent volleys in kittens. Figure 3 illustrates an experiment designed to estimate the proportion of motoneurones in two pools (MG and LGS) which could be fired by single volleys in the respective muscle nerves (Jefferson & Benson, 1953). The monosynaptic reflex discharges produced by maximal afferent volleys in the left MG and LGS nerves were recorded from the central ends of the cut L7 and S1 ventral roots on the left side of the spinal cord (Fig. 3*A* and *E* for the MG reflexes in the S1 and L7 roots, respectively; Fig. 3*C* and *G* for the LGS reflexes). The reflexes were compared with the antidromic spike potentials recorded from the peripheral ends of the cut L7 and S1 ventral roots of the right side of the cord produced by stimulation of the right MG and LGS nerves (Fig. 3*B*, *F* and *D*, *H*). The antidromic spikes were considered to represent the total motoneuronal pools of the MG and LGS nerves. Any dispersion of the reflex spikes would reduce their amplitudes, which would be against the argument. The use of the two sides of the body for this comparison is permissible because of the nearly symmetrical distribution of the motor axons in the comparable roots of the two sides (Jefferson & Benson, 1953; Eccles, Kozak & Westerman, 1962). A control is provided by the use of both the L 7 and S 1 ventral roots, since most of the GS motor axons would pass through one or the other of these roots.

Jefferson & Benson (1953) showed that, in adult cats, the reflex height usually attained the height of the antidromic spike after post-tetanic potentiation; whereas before potentiation only about 20–46 % of the neurones in a motoneuronal nucleus fired an impulse in response to a volley to its muscle nerve. It is significant that in three of the four pairs of records (Fig. 3) the reflex spike was actually larger than the antidromic spike and in the remaining pair (*C* and *D*) the reflex spike was only slightly smaller. The values for the various combinations are as follows (in μV): 765 and 670 for Fig. 3 *A* and *B*; 420 and 226 for *E* and *F*; 95 and

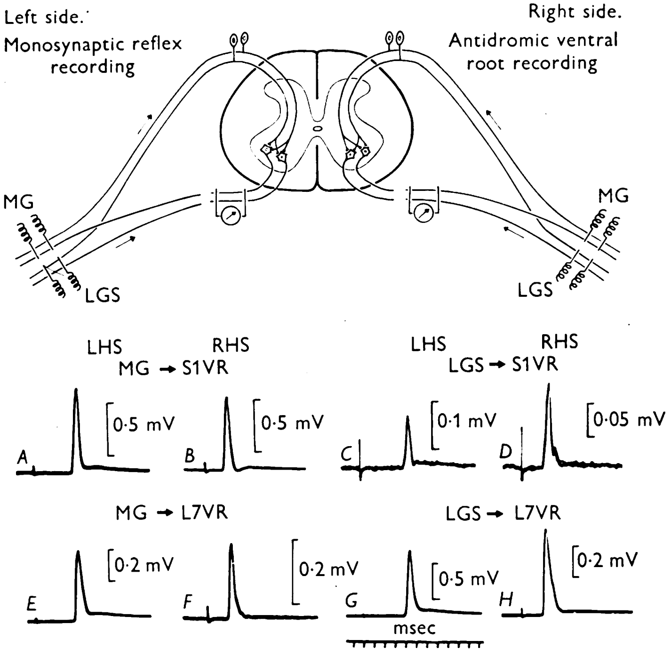

Fig. 3. In a 14-day-old kitten the monosynaptic reflexes on the left side evoked by both LGS and MG into the cut central ends of the L 7 and S 1 ventral roots are compared on the right side to the direct antidromic spike evoked by LGS and MG into the cut peripheral ends of L 7 VR and S 1 VR. The inset indicates the recording conditions on both sides. The MG reflex into S 1 VR (*A*) is compared to the direct axonal discharge MG into S 1 VR on the right (*B*); similarly *E* and *F* for MG into L 7 VR; *C* and *D* for LGS into S 1 VR; and *G* and *H* for LGS into L 7 VR. The reflexes were recorded at a repetition rate of 0·3 c/s and the antidromic ventral root recordings were recorded at 2 c/s. (Alterations in frequency had no effect on the latter unless very high repetition rates (100 c/s) were employed.) Each record has its own potential scale. Same time scale applies for all records.

116 for *C* and *D*; and 855 and 515 for *G* and *H*. Not only must the reflex spikes in kittens include discharges of most, if not all, the motoneurones in the respective homonymous pools, but they must include discharges in other motoneurones as well.

At least some of the motoneurones other than the homonymous ones belonged to the motoneuronal pool of the other branch of triceps surae. This was shown by records made in the same animal before the ventral roots had been cut. Afferent volleys were set up in either the MG or the LGS nerve of one limb and records were made from the other of the pair of nerves. The results are shown in Fig. 4. A volley in the LGS nerve produced a monosynaptic reflex discharge in the MG nerve (Fig. 4*A*) and vice versa (Fig. 4*B*). Heteronymous monosynaptic reflexes were also observed by Wilson (1962) in young kittens, but are rare in adult cats (Lloyd & McIntyre, 1955).

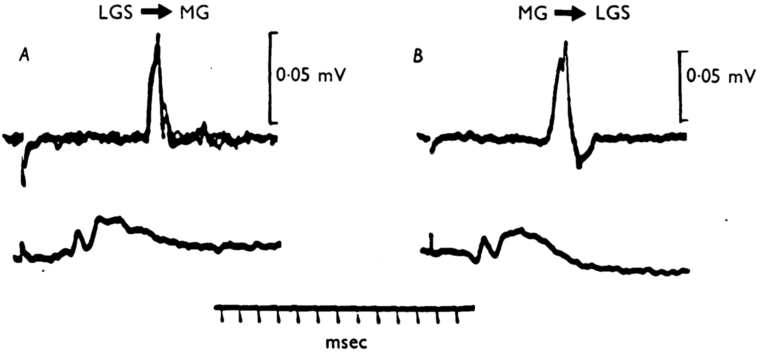

Fig. 4. In a 14-day-old kitten with all dorsal and ventral roots intact the monosynaptic reflex evoked by stimulation of LGS is recorded in the MG nerve (*A*) in the periphery (potential scale = 0·05 mV). The surface recording of the LGS volley is illustrated below. Similarly in *B* the monosynaptic reflex evoked by MG is recorded in LGS nerve at a different potential scale (0·05 mV at the right). Millisecond scale for *A* and *B*. The reflexes were recorded at a repetition rate of 0·3 c/s.

The absence of a subliminal fringe in kitten motoneuronal pools. The apparent lack of a significant subliminal fringe in kitten motoneuronal pools may be correlated with larger monosynaptic EPSPs of kittens relative to adult cats (Eccles *et al.* 1963). For the purpose of that survey, kitten EPSPs were elicited at a repetition rate of 10/sec, which was the rate employed for the study of EPSPs in adult cats (Eccles, Eccles & Lundberg, 1957*a*; Eccles & Lundberg, 1958; Eccles, Eccles & Shealy, 1962). However, the experiments illustrated in Figs. 5 and 6 indicate that this procedure may have led to an underestimate of the size of kitten monosynaptic EPSPs relative to the adult size when low repetition rates are involved.

The technique employed in Figs. 5 and 6 follows that used by Curtis & Eccles (1960) in their study of the effects of repetitive stimulation upon the size of the monosynaptic EPSPs of motoneurones in adult cats. They found that after several successive volleys in Group Ia afferent fibres the evoked monosynaptic EPSPs reached a steady-state amplitude, the value of which depended upon the frequency of stimulation. Similarly, in Fig. 5*A* the upper traces show superimposed monosynaptic EPSPs evoked by maximum Ia homonymous afferent volleys at various frequencies and recorded intracellularly from a GS motoneurone in a 41-day-old kitten. The EPSP was just at threshold for evoking discharges of the motoneurone when the stimulus frequency was 1/sec or less. The amplitude of the EPSP at the steady state decreased as the frequency of stimulation

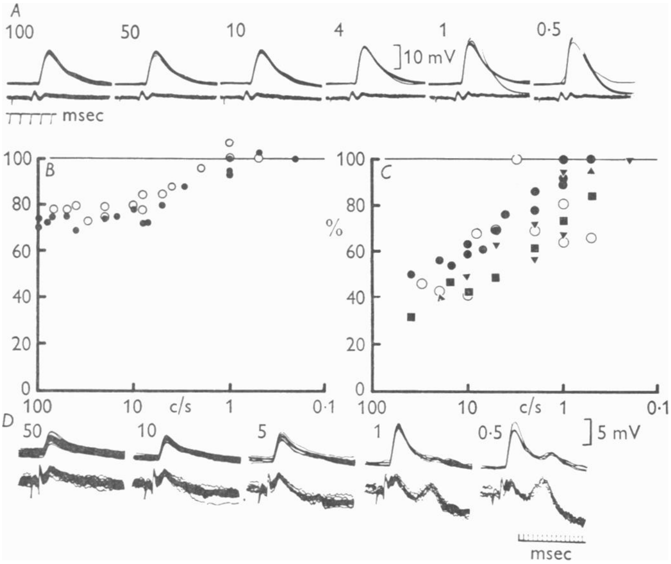

Fig. 5. In *B* are plotted the size of homonymous monosynaptic EPSPs in a 41-day-old kitten evoked in a GS motoneurone (illustrated in *A*) and a PL motoneurone. In *A* the upper traces are some of the EPSPs plotted in *B* at 10 mV potential scale and at different repetition rates given in c/s on each record (open circles, GS neurone; filled circles, PL neurone). In *C* are plotted the size of homonymous EPSPs from a 31-day-old kitten (two GS, triangles and open circles, and SMAB, filled circles) and from a 32-day-old kitten (PBST, squares) at different repetition rates. Examples of the PBST EPSPs (5 mV, potential scale) are given in *D*; the number on each record indicates the repetition rate in c/s. The lower traces in *A* and *D* show the surface recording from the cord dorsum of the entering afferent volley.

increased to 10/sec, as shown in Fig. 5*A* and by the plotted points in the graph of Fig. 5*B* (open circles), and then remained steady with frequencies up to 100/sec. The same pattern of response was seen in a PL motoneurone in that experiment (Fig. 5*B*, filled circles) and in an SMAB motoneurone in another experiment (a 39-day-old kitten, shown in Fig. 6*A*, *B*).

It should be emphasized that the level of the maximum depression of the EPSP in the 41- and 39-day-old kittens (70–80 % of control) was comparable to that reached in adult cats. However, in contrast to the findings in adult animals, there was little or no potentiation of the EPSP when the stimulus frequency was increased to 30–100/sec. In younger animals there was even a progressive decline of the EPSP as the stimulus frequency was raised from 30 to 100/sec. This may be seen for a 32-day-old kitten in the specimen records of Fig. 5*D* for the EPSPs produced in a PBST motoneurone by various rates of stimulation of the PBST nerve. The graph of Fig. 5*C* (filled squares) indicates that the amplitude of the PBST EPSP was depressed to about 40 % of the control height at stimulus

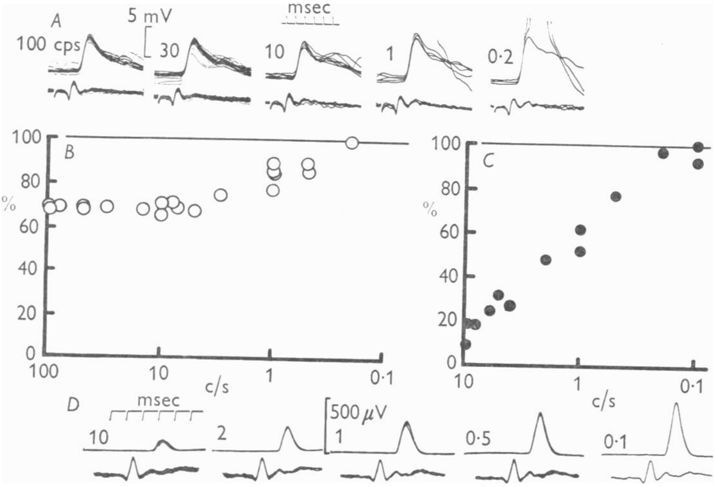

Fig. 6. In a 39-day-old kitten the monosynaptic EPSP evoked by SMAB in an SMAB motoneurone is recorded after reaching a steady state when stimulated at different repetition rates (numbers on each record in *A*). All EPSPs recorded at 5 mV potential scale and msec time scale. In the lower traces in *A* are the records of the afferent volley recorded at the entry of the L 7 dorsal root into the spinal cord. The EPSP size is plotted in *B* as a percentage of the control size (taking the control at 1/2 sec to equal 100 %), against the repetition rate from 1/2 to 100/sec. In *C* from the same animal, the monosynaptic FDHL reflex into L 7 VR is similarly plotted. Examples of the FDHL reflexes are illustrated in *D* at 500 μV potential scale, numbers give repetition rates in c/s. The lower trace represents the entering FDHL volley.

frequencies of 10/sec or above. The EPSPs of three motoneurones in another experiment in a 31-day-old kitten (triangles, open and filled circles) were depressed to nearly the same degree.

It is well established that low frequency repetition of a monosynaptic reflex (between 1/10 sec and 10/sec) results in its depression (Jefferson & Schlapp, 1953; Evanson, 1956; Lloyd & Wilson, 1957; Lloyd, 1957). This depression is explained by the reduction in the size of the EPSP which accompanies comparable rates of stimulation (Curtis & Eccles, 1960). 'Low frequency depression' of kitten monosynaptic reflexes has been described by Wilson (1962), and another example is shown in Fig. 6 *C* and *D*. The FDHL nerve was employed to evoke the monosynaptic reflex, sample records of which are shown for the indicated frequencies of stimulation in Fig. 6 *D*. The amplitudes of the reflex spikes relative to the control size are plotted in Fig. 6 *C* for the various frequencies. The experiment was the same as that illustrated in Fig. 6 *A*, *B*. The size of the reflex declined as well as the EPSP size when the frequency of stimulation was increased from 1/10 sec to 10/sec. A similar relation between reflex size and stimulus rate was seen in the records of Fig. 1, both for the monosynaptic reflex evoked by the conditioning MG volley and for the reflex from the test LGS volley.

The experiments illustrated in Figs. 5 and 6 *A*, *B* suggest that the amplitudes of monosynaptic EPSPs of young kittens would be considerably larger than comparable adult EPSPs when the EPSPs are evoked by low frequency stimulation. Clearly, such large EPSPs could produce discharges in a large proportion of a motoneuronal pool, and they could, if present in heteronymous motoneurones, also account for the monosynaptic reflex discharges into the nerves of synergic muscles (Fig. 4).

DISCUSSION

The evidence obtained in this study suggests that in young kittens maximum group I afferent volleys in muscle nerves evoke monosynaptic reflex discharges from almost all homonymous motoneurones with the consequence that there is very little subliminal fringe for the display of facilitation and post-tetanic potentiation as has been reported by Malcolm (1955), Skoglund (1960*a*, *b*) and Wilson (1962).

The reason for the small or absent subliminal fringe in kittens appears to be the large EPSPs which are produced in their motoneurones by a low-frequency activation of the monosynaptic pathway. There are a number of possible explanations for these large EPSPs. The most obvious is the smaller size of kitten motoneurones than of adult ones (R. M. Eccles and W. D. Willis, unpublished observations). Another explanation is suggested by the pattern of change in the EPSP height with alterations in the frequency of stimulation (Figs. 5 and 6*A*, *B*). In adult cats, the EPSP increases in size as the frequency of stimulation is raised from 5–10/sec to 30–100/sec (Curtis & Eccles, 1960). This is thought to reflect a process of transmitter mobilization, perhaps by a movement of synaptic vesicles closer to the presynaptic membrane (cf. Eccles, 1964). The failure of kitten EPSPs to show this response might indicate a deficiency in the mechanism for transmitter mobilization in immature presynaptic terminals. However, it might equally well reflect a greater transmitter release from the terminals, with a consequently greater transmitter depletion than in adults. The greater transmitter release then might help explain the large EPSPs seen when adequate time is allowed for transmitter replenishment.

It is obviously too early to decide between these and other explanations of the large EPSPs found in kitten motoneurones. Clearly, however, further investigation of the role of development upon synaptic transmission is an important approach to a full understanding of reflex activity.

SUMMARY

1. In kittens, 10–41 days old, the size of the reflex or the monosynaptic EPSP evoked by a volley in a muscle nerve has been examined under different conditions, e.g. repetition rates or responsiveness to a volley in a synergic muscle nerve.
2. The number of motoneurones in the subliminal fringe was shown to be extremely small or absent in very young kittens.
3. In older kittens the monosynaptic EPSPs in their response to frequency, showed a gradual change towards the adult pattern.
4. An explanation in terms of 'transmitter mobilization' in the presynaptic terminals is suggested for the differences between motoneurones in kittens and adult cats.

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