The Functional Significance of Arm Movements in Neonates

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of the *D-mef2* 5' flanking sequence and 0.6 kb of exon 1 was cloned into the P-element transformation vector CaSpeR AUG-β–gal (*31*) to generate a *D-mef2–lacZ* transgene. This sequence was stably introduced into the *Drosophila* genome by P element–mediated germline transformation [G. M. Rubin and A. C. Spradling, *Science* **218**, 348 (1982)]. The yw^{67c23} strain was used for embryo injections, and flies transformed with *D-mef2-lacZ-CaSpeR* were identified by w^+ selection. Similar expression patterns were observed in six independent lines.
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16. Strains *Df(2R)eve*$^{1.27}$*/CyO* and *b Adh cn/CyO*; *ry*^{506} were obtained from the Bloomington stock center; *Df(2R)X1/CyO* was a gift from M. O'Brien and P. Taghert (Washington University Medical Center, St. Louis, MO); *Sp/CyO; ry506Sb P[ry+Δ2–3][99B]/TM6,Ubx* was obtained from W. Mattox (M. D. Anderson Cancer Center, Houston, TX); and *P[lacZ,ry+]2487* was obtained from R. Davis (Baylor College of Medicine, Houston, TX).
17. Isolation of genomic clones and the restriction mapping and Southern (DNA) blot analysis to map the *D-mef2* gene, the deficiency breakpoints, and P-element insertions were performed as described (*32*). Primer extension and ribonuclease (RNase) protections to map the transcription site were done according to the manufacturer's protocol (Ambion), as described (*32*).
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21. The P-element mobilization strategy was based on those described (*20*). Homozygous viable P element, *P[lacZ, ry+]2487*, residing near the *D-mef2* locus was mobilized by introducing the Δ2–3 transposase into the stock. Female flies from this cross, genotype *P[lacZ,ry+]/CyO;ry*^{506}*Sb P[ry+Δ2-3](99B)*, were collected, and the P element was stabilized by crossing out the Δ2–3 transposase by mating to *b Adh cn/CyO; ry*^{506}. Single males with a putative *D-mef2* insertion from the mobilized *P[lacZ,ry+]2487*/CyO;ry*^{506} were screened for lethal mutations over the *Df(2R)X1/CyO* that spans the *D-mef2* locus. Screening of 1000 lines with a potentially transposase-mobilized *2487* P element yielded 15 lines that were lethal in trans to the deficiency. We determined which, if any, of the lethal lines contained a P-element insertion that disrupted *D-mef2* expression by balancing individual lethal lines over *CyO* and screening genomic DNA from the lines by PCR, using a pool of primers from *D-mef2* and the terminal repeat at each end of the P element (*33*). One lethal line, designated P544, was identified in which a PCR product was generated. The *D-mef2* primer that, in combination with the P-element primer, produced the PCR product was identified by performing the reactions with individual primers. Further mapping and sequencing of the genomic DNA from the P544 lethal line showed that ~25 kb of genomic DNA between the original insertion site of the *2487* P element and –320 bp relative to the transcription initiation site of *D-mef2* had been deleted from the *P544* chromosome such that the proximal end of the *2487* P element was inserted in the *D-mef2* promoter. Although it is possible that the *P544* chromosome has other nearby alterations, this is unlikely because it is lethal in trans to several recently identified EMS alleles of *D-mef2* (*22*).
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33. The PCR procedure was carried out as described [K. Kaiser and S. F. Goodwin, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 1686 (1990)], with 1/50th of a fly equivalent for each reaction. The primers were P, 5'-CGACGGGACCACCTTATGTTATTTCA; A, 5'-GGGATGTCAGGTCGCTGGCGAGGTG; and B, 5'-CCAAAGCGATGTTAGCAGGGGTG. The *D-mef2* insertion line *P[lacZ, ry+]544/CyO* was confirmed by flanking primer sets and by Southern blot analysis.
34. Embryos were stained with the indicated antibodies as described (*31*). Antibody dilutions were as follows: anti–D-MEF2, 1:1000; antibody to *Drosophila* muscle myosin (*23*), 1:400; and anti–Fas-III (provided by C. Goodman) (*27*), 1:10. For mutant analysis, embryos were double-stained with either anti–D-MEF2 or anti–β-galactosidase (Cappell) at a dilution of 1:5000, to identify homozygous mutants. The *CyO* balancer chromosome was marked with an *Antp-lacZ* reporter (obtained from J. Botas), allowing identification of heterozygous embryos from homozygous embryos.
35. Rabbits were immunized with a histidine-tagged D-MEF2 fusion protein encompassing amino acids 1 to 472 of D-MEF2, cloned in-frame into the pRSETB vector (Invitrogen). The fusion protein was purified from BL21-LysS cells with the 6XHis/Ni-NTA purification system (Qiagen, Chatsworth, CA).
36. Within the coding region, introns are found at the following positions: intron 2, after codon 18; intron 3, after codon 86; intron 4, after codon 193; intron 5, after codon 226; intron 6, after codon 329; intron 7, after codon 468. The DNA sequence has been deposited in GenBank, accession number U19493.
37. The breakpoints of the different deficiency chromosomes were determined by Southern blot analysis with several probes from the *D-mef2* gene and 5' flanking region and from the P element.
38. Embryos were collected, fixed, and hybridized with digoxigenin-labeled probes (Boehringer–Mannheim), as described previously (*10*). To identify *D-mef2* homozygous mutants, we double-stained embryos for lacZ activity or D-mef2 mRNA in parallel with the individual probes. Probes were obtained from the following sources: Dmyd/nautilus (*28*), apterous (J. Botas), tinman (*25*), bagpipe (*26*), S59 (*30*), and DFR1 (*24*).
39. We are grateful to E. McGuffin and W. Mattox for advice and assistance, S. Galewsky for comments on the genetic screen, R. Davis for the gift of the P2487 stock, and D. Kiehart for anti-MHC. We also thank J. Botas for advice. Supported by grants from NIH, the Muscular Dystrophy Association, the Robert A. Welch Foundation (to E.N.O.), and NSF (to R.A.S.) B.L. was supported by an NIH training grant.

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The Functional Significance of Arm Movements in Neonates

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Arm movements made by newborn babies are usually dismissed as unintentional, purposeless, or reflexive. Spontaneous arm-waving movements were recorded while newborns lay supine facing to one side. They were allowed to see only the arm they were facing, only the opposite arm on a video monitor, or neither arm. Small forces pulled on their wrists in the direction of the toes. The babies opposed the perturbing force so as to keep an arm up and moving normally, but only when they could see the arm, either directly or on the video monitor. The findings indicate that newborns can purposely control their arm movements in the face of external forces and that development of visual control of arm movement is underway soon after birth.

Moving a limb or the whole body in a controlled manner requires acting in conjunction with gravity and other external forces (*1*). This means that movements cannot be represented in any preprogrammed, context-insensitive way (*2*). Accurate control requires on-line regulation of muscular activation on the basis of perceptual information about the dynamics of the limb movement and the external force field, as well as about the movement of the limb relative to objects or surfaces to which it is being guided. Are neonates capable of such perceptuo-motor control, or are their movements to be seen as simply reflexive, showing no evidence of intentionality or control?

To test whether newborn babies between 10 and 24 days take account of external gravitational forces in moving their limbs, we measured spontaneous arm-waving movements while the baby lay on its back with its head turned to one side (*3*). Free-hanging weights, attached to each wrist by strings passing over pulleys, pulled on the arms in the direction of the toes (Fig. 1A). The hand the baby was facing was called the ipsilateral hand; the opposite hand was called the contralateral hand (Fig. 1B).

A typical recording of a newborn baby waving both arms with no weights attached is shown in Fig. 1C. The seen ipsilateral hand shows much movement, whereas the unseen contralateral hand is predominantly stationary with only occasional movement. To test whether newborns need to see their

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hand to move it in the same region despite the force tending to pull it away, we divided the babies into three groups. In group 1, the babies could see only the ipsilateral hand (Fig. 1A). The babies in group 2 were prevented from seeing their arms directly, but were presented with a real-time video image of the contralateral arm on a small monitor placed beside their head. The babies in group 3 had occluders at the sides of the head which prevented them from seeing either arm. The results show that the newborn babies moved a hand up and down in the same place despite the pull of the string on their wrists, but only when they could see the hand, either directly or on the video monitor: When a hand was invisible it was pulled down by the string (Fig. 2). In addition, the amplitude of movement of a seen hand was significantly larger than that of an unseen hand (Fig. 3).

Thus, newborn babies purposely move their hand to the extent that they will counteract external forces applied to their wrists so as to keep the hand in their field of view. In addition, newborns move their arms more when they can see them. These results are in agreement with earlier findings on prereaching (*4*–*6*) and hand-mouth coordination in newborn babies (*7*) and counter the view that neonatal arm movements are purposeless, unintentional, and reflexive and can simply be described as excited thrashing of the limbs (*8*–*10*).

Instead, while watching their moving arms, newborn babies acquire important information about themselves and the world they move in—information babies need for later successful reaching and grasping beginning at around 4 to 5 months. To successfully direct behavior in the environment, the infant needs to establish a bodily frame of reference for action. Because actions are guided by perceptual information, building a frame of reference for action requires establishing information flow between perceptual input and motor output. It also requires learning about bodily dimensions and movement limitations (*11*, *12*). Here vision plays an important role, and many lessons in practical optics have to be learned in the early weeks before reaching for toys can emerge (*13*). Infants have to learn, for example, how long their arms are in order to perceive what is within reach and what is out of reach. It seems likely that a fast growing infant will need constantly to recalibrate the system controlling movement, and visual proprioceptive information is least susceptible to "growth errors" (*14*).

It therefore seems plausible that the spontaneous arm waving of neonates of the kind measured in our experiments helps in the construction of a bodily frame of reference for action. Our findings could have practical implications for babies with visual

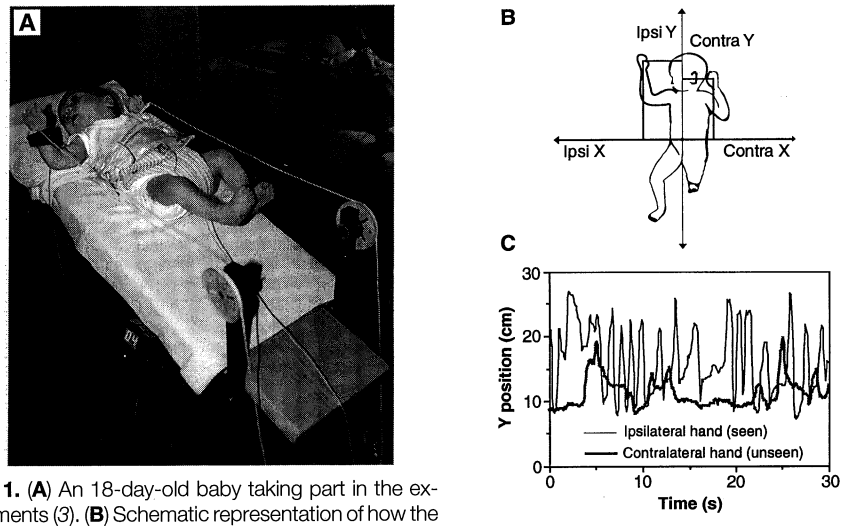

Fig. 1. (**A**) An 18-day-old baby taking part in the experiments (*3*). (**B**) Schematic representation of how the *y* coordinates of the hands were measured. The hand the baby was facing was called the ipsilateral hand; the opposite hand was called the contralateral hand. (**C**) A typical *y* coordinate record of a newborn baby waving both arms without weights attached. The thin line represents the seen ipsilateral hand, the thick line represents the unseen contralateral hand. Each trial yielded two basic measures: the average *y* coordinate, measuring the average position of the hand; and the standard deviation of the *y* coordinate, measuring the amplitude of the movement of the hand.

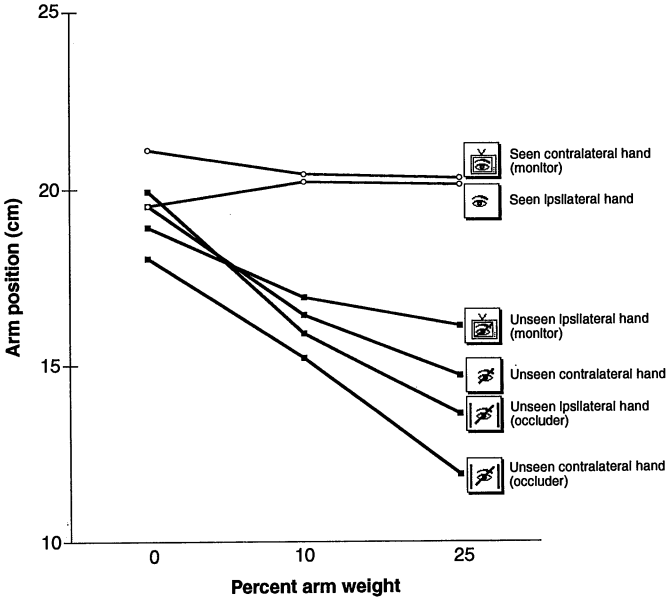

Fig. 2. Means of the average *y* coordinates of the infants' wrists in the no weight, 10% arm weight, and 25% arm weight conditions for the three groups of newborn babies for the seen ipsilateral hand and the unseen contralateral hand (group 1, $n = 5$); for the unseen ipsilateral hand and the seen contralateral hand, visible in real-time on a small video monitor (group 2, $n = 5$); and for the unseen ipsilateral hand and the unseen contralateral hand (group 3, with occluders at the sides of the baby's head, $n = 5$). Each data point represents the mean of 40 trials (eight 30-s trials, $n = 5$). The results show that the newborn babies moved only the hands they could see (that is, the seen ipsilateral hand and the contralateral hand that was visible on the video monitor) up and down in the same place despite the pull of the string on their wrist. In contrast, all unseen hands were pulled down by the string [mixed measures analysis of variance (ANOVA), $F(4,24) = 7.38$, $P < 0.001$].

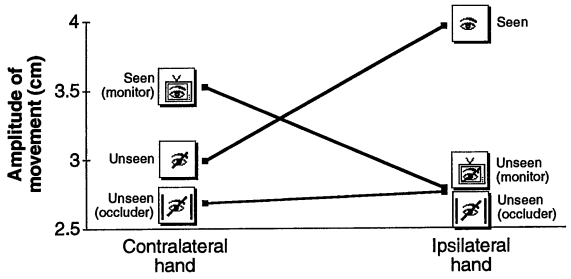

Fig. 3. Mean amplitudes of hand movement (standard deviations of the *y* coordinate of the wrist on trial) in the *y* direction for the three groups of five newborn babies for the ipsilateral hand and the contralateral hand which were, respectively, seen and unseen for group 1, unseen and seen on a video monitor for group 2, and both unseen for group 3. Each data point represents the mean of 40 trials. The amplitudes of movement of the seen ipsilateral hand and the contralateral hand that was visible on the video monitor were significantly larger than those of all the other unseen hands, indicating that the babies moved the hands more when they were able to see them [mixed measures ANOVA, $F(2,12) = 11.93$, $P < 0.002$].

deficits and for the early diagnosis of premature infants at risk of brain damage. If early arm movements have an important function for later reaching skills, then infants with signs of hypoactivity or spasticity of the arms should be monitored closely for retardation in the development of reaching, and possibly other perceptuo-motor skills too. In such cases, early intervention should concentrate on helping the baby to explore its arm and hand movements, both visually (*15*) and nonvisually (*16*).

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3. Infrared light-emitting diodes (LEDs) were fastened onto soft bands around the baby's wrists. The LEDs were viewed by an overhead Selspot camera, with optical axis vertical, from a distance of 1.5 m. The *x* axis in the camera's view was lined up perpendicular to the infant's body axis. The Selspot data were recorded on a computer at 62 frames per second. Strings were attached to the baby's wristbands. The strings passed over pulleys at the foot of the bed and could have weights attached to their ends. The weights used were 0%, 10%, and 25% of the estimated weight of the baby's arm (*17*). With weights added, the string exerted a pull on the baby's wrists approximately parallel to its body axis in the direction of its toes. Each infant was tested for a total of 12 min. The experiment comprised six blocks of four 30-s trials over which the three weights were randomly distributed. A total of 15 newborn babies were randomly assigned to one of the three experimental groups. In group 1 the babies could see the arm they were facing (seen ipsilateral arm, unseen contralateral arm). The babies in group 2 were prevented from seeing their arms directly, but were presented with a real-time video image of the arm they were not facing on a small video monitor placed beside the infant's head (unseen ipsilateral arm, seen contralateral arm). The babies in group 3 had occluders at the sides of the head ("blinkers"), which prevented them from seeing either arm while allowing them to see elsewhere (unseen ipsilateral arm, unseen contralateral arm).
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Potentiation of Transmitter Release by Ciliary Neurotrophic Factor Requires Somatic Signaling

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Neurotrophic factors participate in the development and maintenance of the nervous system. Application of ciliary neurotrophic factor (CNTF), a protein that promotes survival of motor neurons, resulted in an immediate potentiation of spontaneous and impulse-evoked transmitter release at developing neuromuscular synapses in *Xenopus* cell cultures. When CNTF was applied at the synapse, the onset of the potentiation was slower than that produced by application at the cell body of the presynaptic neuron. The potentiation effect was abolished when the neurite shaft was severed from the cell body. Thus, transmitter secretion from the nerve terminals is under immediate somatic control and can be regulated by CNTF.

The differentiation and survival of neurons in the nervous system depend on the action of neurotrophic substances (*1*). Ciliary neurotrophic factor is a protein that promotes the differentiation or survival (or both) of a wide range of cell types in the vertebrate nervous system (*2*). Administration of CNTF to chick embryos reduces naturally occurring motor neuron death (*3*), and mice carrying a null mutation in the CNTF gene show progressive motor neuron atrophy and postnatal neuron loss (*4*). In addition to the long-term trophic effects of CNTF, we report here that CNTF also exerts acute regulatory actions on the synaptic function of developing neuromuscular synapses. The site of CNTF action was examined by local perfusion of CNTF to the synapse or the cell body of the presynaptic neuron in cell cultures. These studies demonstrated that rapid signaling with the cell body is required for the synaptic potentiation induced by CNTF. The secretory function of the presynaptic nerve terminal is thus under an immediate regulation by one or more factors from the cell body, and such somatic regulation can be modulated by CNTF.

Spontaneous synaptic currents (SSCs) were monitored by whole-cell, voltage-clamp recordings (*5*, *6*) from the postsynaptic myocyte in 1-day-old *Xenopus* nerve-muscle cultures (*7*). These currents are produced by spontaneous release of quantal packets of acetylcholine (ACh) from the presynaptic nerve terminal in the absence of action potentials (*8*). Addition of CNTF (final concentration 100 ng/ml) to the culture resulted in a gradual increase in the frequency of SSCs within 10 to 20 min (Fig. 1, A and B). The average SSC frequency (over 5-min periods) was determined at different times after CNTF application. A maximal response was obtained after 25 min of exposure to CNTF, when the SSC frequency had increased to an average level about five times its initial value (Fig. 1, A and B), although a minority of cells failed to respond to CNTF at this concentration (*9*). Unlike the frequency of the SSCs, the mean amplitude of the SSCs remained unchanged. At 25 to 30 min after CNTF addition, the mean amplitude was 93 ± 20% (SEM, n = 9) of that during the control period prior to CNTF addition, similar to that of the control cultures. No significant change was observed in the amplitude distribution (Fig. 1, C and D). The absence of any effect on the SSC amplitude suggests that the action of CNTF was predominantly on the probability of spontaneous quantal ACh release from the presynaptic nerve terminal, rather than on the size of ACh quanta or postsynaptic ACh sensitivity.

The effect of CNTF on impulse-evoked synaptic currents (ESCs) was also examined. Presynaptic neurons were stimulated at the cell body by an extracellular electrode to fire action potentials at a frequency of about 0.05 Hz, and postsynaptic recordings of ESCs were made at different times before and after addition of CNTF (100 ng/ml). An example of one recording and changes in the average ESC amplitude with time for five experiments are shown in Fig. 2. In control cultures not treated with CNTF, repetitive tests of evoked synaptic responses led to a gradual reduction of the mean ESC amplitude, an activity-dependent synaptic depression known to occur at these developing synapses (*10*). In contrast, a significant increase in the ESC amplitude was observed after 10 min in the presence of CNTF (*11*). Because no change was observed for the amplitude of SSCs, the increase in ESC amplitude is likely to result from an increased depolarization-evoked ACh release from the presynaptic nerve terminal (*12*), rather than an increased postsynaptic responsiveness.

To examine the site of CNTF actions on the neuron, we used a pair of perfusion

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