Receptive Properties of Embryonic Chick Sensory Neurons Innervating Skin

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Koltzenburg, Martin and Gary R. Lewin. Receptive properties of embryonic chick sensory neurons innervating skin. J. Neurophysiol. 78: 2560–2568, 1997. We describe a new in vitro skin nerve preparation from chick embryos that allows detailed study of the functional properties of developing sensory neurons innervating skin. Functionally single sensory afferents were isolated by recording from their axons in microdissected filaments of the cutaneous femoralis medialis nerve, which innervates skin of the thigh. A total of 157 single neurons were characterized from embryos [embryonic days 17–21 (E17–E21), n = 115] and hatchlings up to 3 wk old (n = 42). Neurons were initially classified on the basis of their conduction velocity; those conducting below 1.0 m/s were being classified as C fibers and faster conducting fibers as A fibers. The proportions of A and C fibers encountered in embryonic and hatchling preparations were not very different, indicating that myelination and axon growth proceeds quite slowly over the period studied. Afferent fibers that could subserve nociceptive and nonnociceptive functions were identified in the time period studied. Subpopulations of low-threshold myelinated afferent units exhibited rapidly or slowly adapting discharges to constant force stimuli and could have tactile functions. Many afferent fibers responded to noxious heat and were excited and sensitized by exposure to inflammatory mediators, suggesting that they are nociceptors. The behavior of these units changed in several respects over the period studied. The discharge of C fibers to noxious heat increased with age as did their mechanical thresholds. A substantial population of heat-responsive neurons (34% of the A fibers) present in embryos were not encountered in hatching chicks. This indicates that substantial changes in the physiological response properties of sensory afferents occur after hatching. We conclude that this new preparation can be used for quantitative assessment of the receptive properties of developing sensory neurons and has considerable potential for the investigation of factors, such as neurotrophins, that specify and influence the functional phenotype of sensory neurons during embryonic development in vivo.

INTRODUCTION

Over the past 30 years the physiological properties of sensory neurons innervating skin in mammals has been extensively investigated, showing that primary afferents can be classified on the basis of their responses to adequate mechanical, thermal, or chemical stimuli (Meyer et al. 1994; Perl 1992; Perl and Burgess 1973). These studies have demonstrated that the receptive-field properties of afferent neurons are highly correlated with their neurochemical, biological, and neuroanatomic characteristics (Brown 1981; Koerber and Mendell 1992; Lawson 1992; Smith and Frank 1988). The remarkable specificity of their central connections is probably best exemplified by the fact that selective electrical microstimulation of single cutaneous afferents in humans results in elementary sensations that are specific for each subgroup of afferents (Torebjörk et al. 1987). Thus the way that these receptive-field properties develop in relation to other aspects of the sensory neuron phenotype during embryogenesis is therefore of general neurobiological interest.

Only few studies have investigated the physiological properties of developing cutaneous afferents in rats and cats. Myelinated sensory neurons innervating the footpad of neonatal kittens exhibit a profound increase in sensitivity in the first weeks of life (Ferrington et al. 1984; Ferrington and Rowe 1980). Fitzgerald (1987a, b) recorded primary afferent activity at even earlier stages of development in fetal and neonatal rat pups, both before and during myelination of sensory fibers. The main conclusion of these studies was that the majority of primary afferent types in adult animals could already be distinguished at early developmental stages. Because these early experiments were performed in vivo in very small animals, the stimuli used were necessarily qualitative.

In the present investigation we have adopted an in vitro skin-nerve preparation technique previously developed in the rat (Reeh 1986) to record from both myelinated and nonmyelinated fibers of a purely cutaneous nerve in the chick embryos as young as embryonic day 17 (E17) (Hamburger and Hamilton stage 43, HH 43; hatching takes place on day 21) (Hamburger and Hamilton 1951). Besides the clear practical advantages of stable recording conditions and the ability to apply a wide range of controlled quantitative stimuli, this chick preparation is attractive for several other reasons. Because the chick embryo is easy to manipulate early in development, it has been extensively used by embryologists (Hamburger 1980; Le Douarin 1982). For example, much evidence regarding the neurotrophic factor requirements of sensory neurons was first discovered using the chick model (Davies 1994; Levi-Montalcini and Hamburger 1951; Lewin and Barde 1996), and there is an extensive literature describing the biophysical properties of cultured embryonic chick dorsal root ganglion neurons (Gottmann et al. 1988; Nowicki 1992). The few isolated reports describing the physiological properties of primary afferents in the adult chicken indicate that they largely resemble those in mammals (Gentle 1989). The present paper is the first description of the functional response properties of sensory neurons in the developing chick. These results will allow us to eventually relate the differentiation of primary...
afferebent receptive properties to their biophysical, neuroanatomic, and neurochemical phenotype. Thus one can investigate in more detail those factors that govern normal development of primary afferent phenotypes.

**Methods**

**Skin-nerve preparation**

The chick skin-nerve in vitro preparation (Fig. 1) was modified from rat, which has been described in detail previously (Kress et al. 1992; Reeh 1986). In the case of embryonic chicks, a window was first made in the egg above the air sac and the animal decapitated in ovo. Hatching chicks were first killed by CO₂ inhalation followed by decapitation. After clipping and gentle removal of the feathers, the skin of the hindlimb was carefully dissected together with the attached cutaneous femoralis medialis (CFM) nerve. The nerve was dissected free to the crural plexus to ensure a sufficient length of nerve for recording. The preparation was then placed “inside-up” in an organ bath to facilitate oxygenation and drug accessibility through the corium side of the skin (Fig. 1) and superfused (15 ml/min) with an oxygen-saturated modified synthetic interstitial fluid (SIF) solution containing (in mM) 123 NaCl, 35 KCl, 0.7 MgSO₄, 1.7 NaH₂PO₄, 2.0 CaCl₂, 9.5 sodium gluconate, 5.5 glucose, 7.5 sucrose, and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), pH 7.4 ± 0.05 and temperature of 32 ± 0.5°C.

**Recordings**

Using sharpened watchmaker forceps, filaments were teased from the desheathed nerve, and functionally single sensory neurons were recorded extracellularly using a low-noise differential amplifier. Receptive fields of primary afferents were identified with a mechanical search stimulus (manual probing of the skin with a glass rod), which is known to activate >90% of the cutaneous afferents in mammals in this preparation (Kress et al. 1992). Only units with a signal-to-noise ratio of more than 3 were used for further analysis. The conduction velocity of each axon was determined by electrically stimulating the receptive field with supramaximal square-wave pulses (duration 0.1–1.0 ms, interstimulus interval 1–5 s) after insertion of a Teflon-coated steel needle electrode with an uninsulated tip diameter of 10 μm. The median conduction distance of units recorded from embryonic preparations was 25 mm (range 12–50 mm) and in hatching preparations 32 mm (range 17–55 mm). To ascertain the identity of the naturally and electrically evoked activity, a “marking” procedure (Fig. 1) was routinely carried out (Kress et al. 1992). The latency of action potentials of unmyelinated and thin myelinated afferents is stable when regularly stimulated with supramaximal electrical stimuli. However, when the same unit is activated by a short natural stimulus, e.g., mechanical probing, in the interval between two electrical stimuli, there is a characteristic latency shift, which recovers gradually over several seconds (Iggo 1958; Schmelz et al. 1995).

**Mechanical stimulation**

The mechanical threshold of each unit was determined with calibrated von Frey monofilaments with uniform tip diameter of 0.8 mm. The weakest von Frey filament used in this study exerted a bending force of 1 mN because smaller monofilaments were incapable of reliably penetrating the surface tension of the bathing solution. Where possible, each unit was subjected to a standard protocol of stimuli consisting of mechanical, thermal, and chemical stimuli. Constant force stimuli were applied with a feedback-controlled probe with a tip diameter of 0.8 mm placed perpendicularly onto the most sensitive spot of the receptive field. Each stimulus began with an adaptation period of 5 s using a force of 1 mN after the probe had made contact with the skin. In very sensitive units this minimal force could evoke a discharge that in most cases settled within 5 s. Then the force rose within 200 ms to a preset 10-s-long force plateau that varied between 5 and 300 mN after which it returned to the adaptation force of 1 mN for 5 s before the probe was lifted off the tissue. In pilot experiments we determined that this stimulus configuration was suitable for clearly differentiating the adaptation properties of the afferents. Stimuli were delivered in ascending order every minute. Longer interstimulus intervals might have been advantageous to completely avoid interstimulus interaction. However, in initial detailed recordings we determined that desensitization was most noticeable when a high force stimulus was followed by a very low force stimulus and that most of this interference could be observed within 30 s. Thus the protocol used in the present study was a practical compromise between relatively little interstimulus interaction and a desirable speed of data acquisition.

**Thermal and chemical stimulation**

At the end of the series of mechanical stimuli, the neurons’ receptive field and its surroundings were isolated with a self-sealing metal ring (6–12 mm diam), the fluid within the ring was manually removed with a syringe, and a thermocouple was gently applied to measure intracutaneous temperature. Cold stimuli were delivered by giving a 10-ml bolus injection of ice-cold SIF solution that resulted in a temperature nadir of 4–6°C, after which the tempera-
Conduction velocities of natal pups cannot be used as in the adult to separate unmyelinated fibers from myelinated. Since all conduction velocities of the single units recorded fell well into the cutoff values as defined by the compound action potential of afferents conducting slower than 0.7 m/s (indicated with an arrow). The fastest but smallest compound action potential representing A-fiber afferents conducted at a maximal velocity of 5.5 m/s. Distributions of the conduction velocities of single units recorded from embryonic (E17–E21) and posthatching (4–21 days; C) chicks. Fibers conducting at <1.0 m/s were considered to be unmyelinated and shown in black. Note that in the older animals the fastest afferents increase their conduction velocities slightly, but the overall distribution is very similar.

**Results**

**Conduction velocities**

A total of 13 chicken embryos (between E17 and E21) and 7 hatching chicks between 4 and 21 days posthatching were used in this study. Measurement of compound action potentials from the whole nerve was used to estimate the conduction velocity range of axons within the CFM nerve, and a representative example of such record from an embryonic preparation is shown in Fig. 2. The slowest compound potential began between 0.6 and 0.8 m/s and generally had the largest amplitude. The conduction velocity of the fastest potential started at 5–6 m/s and was usually small in amplitude. A third compound was often present with intermediate values for both conduction velocity and amplitude. Since <5% of the axons in cutaneous nerves of the chick are myelinated before hatching (Saxod and Bouvet 1982), the

**Figure 2.** A: several times superimposed sweep of the compound action potential after supramaximal electrical stimulation of the CFM nerve. The largest and slowest conducting peak represents afferents conducting slower than 0.7 m/s (indicated with an arrow). The fastest but smallest compound action potential representing A-fiber afferents conducted at a maximal velocity of 5.5 m/s. Distributions of the conduction velocities of single units recorded from embryonic (E17–E21) and posthatching (4–21 days; C) chicks. Fibers conducting at <1.0 m/s were considered to be unmyelinated and shown in black. Note that in the older animals the fastest afferents increase their conduction velocities slightly, but the overall distribution is very similar.

**Data analysis and statistical tests**

All data were collected with custom-made data acquisition software running on a PC, and action potentials were subsequently analyzed with a template matching program (Forster and Hardwerker 1990). Further quantitative analysis of the recorded neural responses was carried out with custom-designed software. For construction of the stimulus response functions for mechanical stimulation, all action potentials were counted that were recorded in a time period of 11 s after the onset of a force stimulus. This time window was chosen because it contained all spikes during the rise time, the plateau, and the discharge during the release of the stimulus. Stimulus response functions for heat responses were calculated by averaging the discharges of all fibers tested and calculating a linear regression of the running average of this histogram for the time of the temperature increase (Koltzenburg et al. 1992). A unit was scored as chemically sensitive when the average discharge exceeded 0.1 impulses/s for the duration of the superfusion.

All values are given as means ± SE and interquartile range between the 25th and 75th percentile. Appropriate statistical tests were applied after fulfillment of necessary prerequisites (Sachs 1978) using the Statistica software package by Statssoft. Where P values are stated, they are always based on two-tailed distributions.
neurons. rather reflects changes in the receptive apparatus of sensory changes in myelination or conduction velocity of axons, but means that functional changes of the receptive properties of the time period studied here (Saxod and Bouvet 1982). This myelin thickness of Aβ fibers increases only slightly during slowly and is not complete until well into adulthood (Saxod and Bouvet 1982). Consistent with the small alterations of slow myelination in chicken proceeds, in contrast to postnatal mammals, very slowly and is not complete until adulthood (Saxod and Bouvet 1982). Consistent with the small alterations of conduction velocity shown in the present study, the diameter of unmyelinated axons does not change, and axon size and myelin thickness of A fibers increases only slightly during the time period studied here (Saxod and Bouvet 1982). This means that functional changes of the receptive properties observed in the present study cannot be explained by major changes in myelination or conduction velocity of axons, but rather reflects changes in the receptive apparatus of sensory neurons.

Functional classification of units recorded

After identification of the receptive field with a mechanical search stimulus, neurons from both embryonic and hatchling preparations were further characterized by their responses to quantitative mechanical, thermal, and chemical stimuli (Figs. 3 and 4). C fibers generally exhibited a slowly adapting discharge to sustained mechanical stimuli (Figs. 3A and 4). Many of these fibers also responded to thermal and chemical stimulation (Fig. 3B). Whereas most C fibers had slowly adapting mechanosensitive properties, 41% of the A fibers (n = 49 tested) in embryonic or hatchling chick were rapidly adapting and discharged only at the onset or offset of the stimulus (Fig. 4). Quantitatively, these rapidly adapting responses were relatively uniform across a series of increasing force stimuli, presumably because the rise time of the force ramp (200 ms) was kept constant (Fig. 4). The remaining slowly adapting A fibers exhibited a proportional increase in discharge with increasing stimulus strength.

As determined with calibrated von Frey hairs, A fibers (1.0 mN, quartile range 1.0 mN, n = 51) had significantly (P < 0.001, U test), lower mechanical thresholds than C fibers (2.8 mN quartile range 1.0 mN, n = 45) in the embryonic chick (Fig. 5A). In preparations from hatching chicks, there was a significant increase in the von Frey thresholds of both A fibers (2.4 mN, quartile range 1.0 mN; n = 21, P < 0.05, U test) and C fibers (median 5.7 mN, quartile range 4 mN, n = 21; P < 0.01, U test).

The mechanical sensitivity of a subpopulation of the embryonic A and C fibers was tested in more detail by constructing stimulus response functions using a range of supra-threshold stimuli (from 5 to 300 mN). The average stimulus response functions of both slowly adapting A and C fibers increased monotonically between 5 and 100 mN and then reached a plateau (Fig. 5B). However, the discharge evoked in A fibers was consistently higher than that in C fibers (F1,210 = 19.3, P < 0.001, analysis of variance).

Responses to nonmechanical stimuli

Thermal and chemical stimuli were used to further classify the neurons. It is clear that on the basis of mechanical responsiveness alone it would be difficult to unequivocally differentiate between nociceptive and nonnociceptive primary afferents. In mammals virtually all mechanosensitive C fibers recorded in vitro are nociceptors (Kress et al. 1992), but a large fraction of the myelinated fibers fulfill nonnociceptive

![FIG. 3. Examples of C-fiber units recorded from embryonic chick. A: response of a unit to increasing constant force stimuli. This unit was subsequently tested for a response to noxious cold, heat, and inflammatory mediators but did not respond and was consequently classified as mechanosensitive C fiber (CM fiber). B: response of a mechano-heat-sensitive C fiber (CMH-unit, mechanical response not shown) during 2 noxious heat stimuli before and after exposure to inflammatory mediators (10 μM of each bradykinin, serotonin, histamine, and prostaglandin E2). Note that the unit displays a vigorous response to chemicals and an increased response to the 2nd heat stimulus indicating sensitization.](https://www.physiology.org/jn/dt TOC/1984_A.google)
TABLE 1. Proportion of C and A fibers from embryonic chick responding to thermal and chemical stimuli

<table>
<thead>
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<th>Thermal Response</th>
<th>Response to Mediators</th>
<th>Sensitized to Heat</th>
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<tr>
<td>CMH</td>
<td>32% (12/38)</td>
<td>60% (6/10)</td>
<td>80% (8/10)</td>
</tr>
<tr>
<td>CMHC</td>
<td>8% (3/38)</td>
<td>66% (2/3)</td>
<td>66% (2/3)</td>
</tr>
<tr>
<td>CM</td>
<td>60% (23/38)</td>
<td>21% (4/19)</td>
<td>21% (4/19)</td>
</tr>
<tr>
<td>AMH</td>
<td>36% (12/33)</td>
<td>50% (6/12)</td>
<td>42% (5/12)</td>
</tr>
<tr>
<td>AMC</td>
<td>9% (3/33)</td>
<td>0% (0/2)</td>
<td>0% (0/2)</td>
</tr>
<tr>
<td>AM</td>
<td>55% (18/33)</td>
<td>0% (0/18)</td>
<td>28% (5/18)</td>
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The numbers of neurons tested for thermal and chemical responses are shown; 38 A and 33 C fibers were tested for their response to a standard noxious heat or noxious cold stimulus. On the basis of their responses to these stimuli, they could be classified into CMH or AMH (mechano-heat sensitive), into CMHC (mechano-heat and cold sensitive), into AMC (mechano-cold sensitive) or into CM or AM (only mechanoreceptive). The numbers and proportions of these receptor types are listed in Thermal Response. In addition, subpopulations of these receptor types were further tested for a response to algogenic chemicals (Response to Mediators) and for the ability of these chemicals to sensitize the neuron to a 2nd heat stimulus or to induce a de novo response to heat (Sensitized to Heat).

FIG. 5. A: plot of the cumulative mechanical threshold distribution determined with calibrated von Frey hairs for A and C fibers recorded in embryonic preparations. Distributions were obtained from 45 C fibers and 51 A fibers. B: stimulus response functions of slowly adapting A or C fibers recorded in embryonic preparations. Data were obtained from between 6 and 29 fibers per stimulus for A fibers; some fibers were not tested at all stimulus strengths, especially the strongest stimuli for fear of damaging the fibers. For C fibers each point represents the mean of between 10 and 29 fibers; error bars are standard error of the mean.

functions (Airaksinen et al. 1996; Koltzenburg et al. 1997). Because responses to noxious heat or algesic chemicals are known to be a unique characteristic of nociceptors (Handwerker and Reeh 1991; Meyer et al. 1994), we used these stimulus modalities as a tool to ascertain the nociceptive function of units (Fig. 3).

C fibers

Many of the 38 C fibers tested in preparations from embryonic chick responded to a nonmechanical stimulus (summarized in Table 1). Thus 32% of the neurons were excited by noxious heat and were classified as mechano-heat-sensitive C fibers (CMH fiber), and another 8% responded to both noxious heat and cold (‘‘mechano-heat-cold sensitive,’’ CMHC fiber). Superfusion of the receptive field with a mixture of inflammatory mediators (10 μM bradykinin, serotonin, histamine, and prostaglandin E₂) excited 60% of the tested CMH fibers as defined by a discharge exceeding 1 impulse/10 s, and 80% showed an increased heat response thereafter (Fig. 3). Moreover, 21% of the C fibers that did not initially respond to heat did so after exposure to inflammatory mediators. Thus a large proportion of the embryonic C fibers respond to nonmechanical modalities, which is consistent with the view that these neurons subserve a nociceptive function.

Among 33 embryonic A fibers tested, 15 (45%) also responded to noxious heat, cold, or chemical mediators (36% AMH and 9% AMC summarized in Table 1). There was no significant difference between the proportions of embryonic A fibers responding to noxious heat or chemicals (Table 2).

TABLE 2. Proportion of C and A fibers from hatchling chick responding to thermal and chemical stimuli

<table>
<thead>
<tr>
<th></th>
<th>Thermal Response</th>
<th>Response to Mediators</th>
<th>Sensitized to Heat</th>
</tr>
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<tbody>
<tr>
<td>CMH</td>
<td>44% (7/16)</td>
<td>71% (5/7)</td>
<td>66% (4/6)</td>
</tr>
<tr>
<td>CMC</td>
<td>12% (2/16)</td>
<td>0% (0/2)</td>
<td>0% (0/2)</td>
</tr>
<tr>
<td>CM</td>
<td>44% (7/16)</td>
<td>29% (2/7)</td>
<td>29% (2/7)</td>
</tr>
<tr>
<td>AMC</td>
<td>12% (2/16)</td>
<td>0% (0/2)</td>
<td>50% (1/2)</td>
</tr>
<tr>
<td>AM</td>
<td>88% (14/16)</td>
<td>0% (0/14)</td>
<td>7% (1/14)</td>
</tr>
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Same specifications as Table 1; refers only to units tested from preparations taken from hatchling chicks.
In this study we have characterized the receptive properties of sensory neurons innervating the hindlimb skin of chick embryos and in posthatching chicks up to 3 wk old. By using an in vitro preparation and a range of controlled, quantitative stimuli, we found that subpopulations of sensory neurons can readily be identified on functional grounds late in embryonic development. We also show that the response properties are not completely mature, because some response properties change during the early posthatching period.

The subtypes of afferents that emerged were polymodal C fibers, mechanosensitive C fibers, polymodal nociceptive A fibers, and A-fiber mechanoreceptors with rapidly or slowly adapting properties. In mature animals it is usually possible to differentiate nociceptive and nonnociceptive primary afferents on the basis of their different stimulus response functions to mechanical stimuli and conduction velocity alone (Meyer et al. 1994). However, in the developing chick this is not easily accomplished because the distribution of mechanical thresholds and conduction velocities of all fibers spanned a narrow range. This difficulty in differentiating between neurons having different functions applies particularly to A fibers, which are known to contain several subpopulations of nonnociceptive receptors in the adult chicken (Gentle 1989). However, when nonnociceptive stimuli, notably noxious heat and algesic chemicals, are used as diagnostic tools (Handwerker and Reeh 1991), it becomes clear that a large percentage of C fibers as well as A fibers have nociceptive properties. Furthermore, among the A-fiber population, it is possible to differentiate between fibers with rapidly or slowly adapting properties, but further subdivisions were not possible because skin appendages (different kinds of feathers) could not be stimulated selectively due partly to the "inside-up" position of the skin.

In similar preparations taken from adult mice or rats, we have previously shown that the afferent types previously described in vivo can also be easily distinguished using this in vitro inside-up preparation (Airaksinen et al. 1996; Koltzenburg et al. 1997; Kress et al. 1992). It is possible that in the present experiments more information could have been obtained regarding afferents selectively activated by mechanical displacement of the feathers. However, the range of stimuli that we have used here, especially those pertaining...
to nociceptive responses, was most appropriate for the inside-up preparation. Thus the heat stimulus was applied from the epidermal side (from underneath the transparent chamber), and chemicals were applied to the inside face of the skin where such inflammatory mediators would normally accumulate. It has previously been shown in adult rat that the resulting temperature from our standard heat stimulus is ~5°C degree lower when it is measured on the corium side as compared with the epidermal side (Reeh 1986). This fully explains the seemingly lower heat thresholds of nociceptors in vitro compared with the values found in vitro where thresholds are usually measured as skin surface temperature. The quantitative mechanical stimuli used (calibrated von Frey hairs or feedback-controlled constant force stimuli) have also been shown in previous experiments to yield qualitatively identical responses with the exception that thresholds obtained in vitro are systematically lower and less variable than in vivo (Kress et al. 1992; Reeh 1986). This can probably be explained by the lack of a “damping” effect of the epidermis and the homogenous consistency of the silicone elastomer (Sylgard) compared with the various tissues (bone, fat, muscle) underlying the skin in vivo (Kress et al. 1992). In short the inside-up preparation offered the best compromise in allowing a wide range of different stimuli to be appropriately applied to the receptive surface in vitro.

In contrast to the postnatal development of mammals (Fitzgerald 1987b; Fulton 1987), changes in conduction velocity and growth of axons and their myelin sheaths are not prominent in the developing chick during the period investigated here (Saxod and Bouvet 1982). This means that the functional alterations observed in the present study probably reflect changes in the intrinsic receptive properties of the neurons rather than changes in the sampling of neurons due to increased axon growth and myelination. During the 3 wk after hatching, the mechanical thresholds of both A-fiber and C-fiber afferents increased, and median threshold doubled in both populations compared with the embryonic condition. In the adult chicken, the available evidence suggests that mechanical thresholds of nociceptors are even higher (Gentle 1989). Previous studies in rat indicated that thresholds of mechanical thresholds of nociceptors do not appear to increase during the fetal and early postnatal period (Fitzgerald 1987a,b). However, the values recorded in those studies are well below those of the adult rat (Kress et al. 1992; Lewin and Mendell 1994), implying that the receptor threshold rises slowly postnataally. This is in agreement with behavioral measurements that show a slow increase in the mechanical withdrawal thresholds of rats as they mature (Fitzgerald et al. 1988; Lewin et al. 1993). Although this increase in threshold is consistent with a maturation of the intrinsic properties of the afferents, changes in the physical properties of the skin cannot be completely ruled out. However, we think this less likely, first because tissue compliance in this preparation is primarily determined by the elasticity of the Sylgard underneath the skin, and second because changes of tissue compliance play only a minor part even in the response characteristics of low-threshold mechanoreceptors (Baumann et al. 1986; Pubols 1982).

Another functional maturation that we have observed is an increase in the discharge to suprathreshold noxious heat stimuli. This was indicated by an increased slope of the average response to a standard heat ramp in hatching chicks compared with late embryonic chicks (Fig. 6). This occurred in the absence of a significant change in the proportion of unmyelinated afferents that responded to heat stimuli, indicating that the heat-sensitive apparatus within individual neurons is maturing as the animal ages. The increased heat response cannot be assigned to changes in the capacity of the skin to conduct the heat stimulus because the temperature was feedback-controlled at the receptive field (see METHODS). The characteristics of the heat response in chicks were different from those in most mammals. In mammals afferents usually stop discharging at the end of a linearly rising heat ramp when the temperature passes its zenith (Kress et al. 1992). In chick, however, afferents remained active after termination of the heat stimulus as the skin passively cooled. In spite of the marked increase of the nociceptor discharge in the posthatching period, these characteristics appeared not to be different between preparations taken from embryonic or hatching chicks.

We have also shown that afferents from both embryonic and hatching afferents can be activated by a mixture of algogenic chemicals and show an enhanced heat response after chemical stimulation (Fig. 3). This plastic process of nociceptor sensitization, which is an important cause of the increased pain sensations after tissue damage, was very similar to that observed in mature mammalian preparations (Kessler et al. 1992; Koltzenburg 1995; Meyer et al. 1994). In embryonic chicks, we found that 36% of the A fibers (Table 1) responded to noxious heat, but none in the hatching chicks, which is in agreement with results from adult chickens where myelinated trigeminal nociceptors were found not to respond to noxious thermal stimuli (Gentle 1989). The lack of polymodal A-fiber nociceptors in the hatching chicks is especially surprising because the total sample of these faster conducting afferents in the hatchling animals (50%) was not very different from that seen in embryos (53%). This could mean that a population of polymodal nociceptive A fibers disappeared in the immediate posthatching period. Since cell death is thought not to be predominant in the posthatching period (Hamburger et al. 1981), our data are consistent with the idea that a subpopulation of myelinated afferents lose their ability to respond to heat. This suggests that, at the time of hatching, there is still considerable plasticity in the capacity of neurons to respond to noxious stimuli. This is not to say that the A-fiber nociceptors do not exist in the hatching because actually many of these afferents have relatively high von Frey thresholds (40% have thresholds >4 mN).

Much recent work indicates that during development as well as in adult animals the availability of nerve growth factor (NGF) can affect the expression of nerve sensitivity in nociceptors. Application of exogenous NGF during the early postnatal period results in a permanent increase of heat sensitivity of nociceptive afferents found in the adult rat (Lewin and Mendell 1994). Moreover, removal of endogenous NGF in adult animals using a recombinant trkA-IgG fusion protein results in a differential decrease of the nociceptors’ responsiveness to heat and chemicals, but not to mechanical stimuli (Koltzenburg et al. 1996). Furthermore, acute application of NGF to the receptive field of adult ro-
We have been able to characterize in detail the development of inward currents in chick sensory and autonomic neuronal afferents during late embryonic development and the post-hatching period. Our data indicate that several subtypes of afferents can be recognized within several days after they form their central connections. However, the receptive properties of nociceptors undergo gradual maturation in the post-hatching period. Thus the responses of C fibers to noxious heat become more dynamic and robust, and their mechanical thresholds for activation rise gradually as development proceeds. On the other hand A-fiber nociceptors appear to lose their capacity to respond to noxious heat during the same time period.

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