

Fiber Types of the Intrinsic Whisker Muscle and Whisking Behavior

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Some rodent species show rhythmic bouts of vibrissal protractions and retractions, referred to as whisking, that are among the fastest movements performed by mammals. To better understand the muscular basis of whisking, we compared (1) whisker movements of two whisking species (mouse, rat) and a non-whisking species (guinea pig), (2) the muscle fiber composition of intrinsic whisker muscles of whisking and a non-whisking species, and (3) the muscle fiber composition of intrinsic whisker muscles and of selected skeletal muscles. Using high-speed videography, we found that mice, rats, and guinea pigs can generate fast and large-amplitude whisker movements. Guinea pigs do not show bouts of fast, strictly rhythmic whisker movements, and the average speed of their whisker movements is much lower than in mice and rats. Analysis of mRNA expression of myosin heavy chain isoforms, myofibrillar ATPase staining, and antibody labeling indicate that in all three species intrinsic whisker muscles are composed predominantly of type 2B muscle fibers. Intrinsic whisker muscles of mice consisted of type 2B ($\geq 90\%$) and type 2D fibers. In rats we observed, in addition to type 2B/2D fibers, $\sim 10\%$ of slow type 1 fibers, and in guinea pigs we observed $\sim 3\%$ of slow type 1 fibers and 20% of type 2A fibers. Type 2B fibers have high levels of anaerobic glycolytic enzymes providing a rapid source of ATP and high maximum velocity of contraction but are less fatigue resistant than other muscle fiber types. The high percentage of type 2B fibers distinguishes the intrinsic whisker musculature from skeletal muscles and may have evolved for fast scanning of the sensory environment.

Key words: whisker; vibrissas; whisking; muscle fiber; barrel cortex; myosin heavy chain

Introduction

In rodents, the facial vibrissas function as important tactile sensors (Vincent, 1912; Gustafson and Felbain-Keramidas, 1977; Brecht et al., 1997). As in other sensory systems, active positioning of sensors plays an important role in vibrissal function, and in most rodents, facial whiskers can be moved. However, the degree to which whiskers are mobile differs among rodents. Some species like rats, hamsters, and mice display a highly specialized pattern of whisker movements referred to as whisking (Welker, 1964; Lee and Woolsey, 1975; Wineski, 1985; Carvell and Simons, 1990). Whisking behavior is strictly rhythmic and occurs at high repetition rates up to 25 Hz. It can involve large amplitude whisker movements ($>100^\circ$) and occurs mainly in the plane of the whisker row. Thus, whisking is a high-speed behavior with repetition rates that exceed those of most other behaviors displayed by mammals.

Movements of whiskers are generated by two classes of muscles. One class, the intrinsic whisker muscles (also referred to as vibrissal capsular muscle), have their points of attachment completely within the mystacial pad and form a sling around each follicle (Dörfl, 1982). Intrinsic whisker muscles constitute the major share of the whisker pad musculature, and their contrac-

tion causes protraction (forward movement) of the vibrissas (Carvell et al., 1991; Berg and Kleinfeld, 2003). A second class of muscles, the extrinsic muscles, anchors external to the mystacial pad, and their contraction results in retraction (backward movement of the vibrissas) (Wineski, 1985; Berg and Kleinfeld, 2003).

A variety of muscle fiber types can be distinguished in skeletal muscle, and the properties of different muscle fiber types are correlated to the cellular (ultrastructure, mitochondria, etc.) and molecular (myosins, metabolic enzymes, etc.) makeup of the respective fibers. The heavy chain portion of the myosin molecule that determines the major functional characteristic of myosin isoforms provides a particularly useful molecular marker for muscle fiber type (Kelly and Rubinstein, 1994; Weiss et al., 1999). The ATPase activity of various myosin heavy chain (MyHC) isoforms can be differentiated and visualized by histochemical staining procedures (Brooke and Kaiser, 1970) or antibody labeling (Hughes et al., 1993). The presence of different MyHC isoforms correlates well with the functional characteristics of the respective fiber type (Pette and Staron, 1990; Staron, 1997). Thus, fiber types are classified according to the MyHC: type 1 with MyHC1, type 2A with MyHC2A, type 2D with MyHC2D, and type 2B with MyHC2B.

Muscle fibers differ in the stability and fatigue resistance of their contractions as well as in the maximum velocity of contraction. Type 1 fibers, so-called slow fibers, generally have high levels of aerobic oxidative enzymes. Type 1 fibers, therefore, show stability and fatigue resistance from a long-lasting supply of ATP derived from oxidation of nutrients provided by the blood. Type

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2 fibers, so-called fast fibers, can be subdivided into three types. Type 2A fibers have oxidative enzymes and are thus fatigue resistant. Type 2B and 2D fibers have high levels of anaerobic glycolytic enzymes providing a rapid oxygen-independent source of ATP, but this pathway rapidly depletes glycogen stores, and fatigue develops after brief usage (Hughes et al., 1999). The maximum velocity of contraction (V_{max}) for fibers composed of a single MyHC increases in the order $1 < 2A < 2D < 2B$ (Bottinelli, 1991).

Here, we try to identify structural specializations of whisker musculature that are associated with high-speed whisking behavior. Specifically, we asked the following questions: (1) How do vibrissa movements of whisking (mouse, rat) and non-whisking (guinea pig) species compare? (2) What is the fiber composition of intrinsic whisker musculature? (3) How does the fiber composition of intrinsic whisker musculature differ between whisking and non-whisking rodent species? (4) How does the fiber composition of intrinsic whisker musculature compare with the muscle fiber composition of skeletal muscles?

We found that whisker movements are mediated by a specialized musculature that is distinguished from skeletal muscles by a predominance of fast contracting, fast-fatigable type 2B muscle fibers.

Materials and Methods

Animals. The histology of whisker muscles was based on C57/BL6 mice (11–12 weeks old; $n = 10$), wistar rats (4–5 weeks old; $n = 6$), and guinea pigs (10–12 weeks old; $n = 2$) killed by CO₂ inhalation. The soleus and the extensor digitorum longus (EDL) muscles were carefully separated from the surrounding tissue, whereas intrinsic whisker muscles of mystacial vibrissas were dissected out together with the complete whisker pad. The soleus muscle is a hind limb muscle that extends the tarsus; it is a muscle known to be involved in the generation of mainly slow movements. The EDL muscle is a hind limb muscle that extends the second, third, fourth, and fifth digit; it is a muscle known to be involved in the generation of mainly fast movements. The intrinsic whisker muscles are involved in vibrissa protraction as explained in detail below.

Videography of whisking motion based on reflex foil labels. Whisker movements were recorded in freely moving animals ($n = 10$ mice, $n = 6$ rats, $n = 2$ guinea pigs). Whisker movements were documented by a videography technique that allowed sampling at a rate of 150 Hz based on an infrared stroboscope, coupled with an infrared-sensitive video camera. Individual whiskers were labeled with reflex foil to improve contrast. This videography technique was developed to record the movements of single whiskers. Single whisker movement tracking poses three problems: (1) the whiskers move at high velocities requiring short exposure times and a fast recording technique; (2) the whiskers are very thin and offer little optical contrast; and (3) within the 30 whiskers of the array, it is difficult to identify and follow the movements of single whiskers. Very fast recordings with high light gain can be achieved with reflex foil (Junger and Varju, 1990). The reflex foil (7610 High Gain; 3M, Neuss, Germany) is made up of a reflecting sheet of metal loaded with miniature glass balls. The glass balls act as lenses; the net effect of the foil is to reflect light back to its source. Thus, an infrared light-emitting diode (LED) was placed laterally as a light source, and its light was reflected onto the scene by a 45° inclined half-silvered mirror. The video camera was placed above, videotaping through the mirror. The video camera had a frame rate of 50 Hz (i.e., a 20 msec exposure time for each frame). The frame onsets were extracted from the video signal, and for each frame three 1 msec flashes of the LED were triggered at 6.67 msec intervals. This led to a stroboscopic image of whisker movements with three whisker positions in each frame, leading to an effective sampling rate of 150 Hz. With adjustment of the focal length of the camera to match the virtual position of the light source in the mirror, the light gain can be maximized. Individual whiskers were marked with a small patch of reflex foil (0.3 × 1.5 mm). Adhesive-backed reflex foil patches could be mounted easily on

vibrissal shafts for 2- to 10-min-long periods. Even very small patches of reflex foil provided good reflections. This permitted the videotaping of movements of single vibrissas in light or in total darkness purely under infrared illumination.

To determine whether the foil label affected the movements of the labeled whisker, we performed multi-whisker-labeling experiments, in which we labeled two whiskers at different distances from the whisker origin and with different sized foil patches. It has been established that during whisking in air all whiskers engage in very similar movements (Welker, 1964; Carvell and Simons, 1990; but see also Sachdev et al., 2002). When foil patches alter whisker movements, this should result in a deviation of the whisker with the larger and more distant foil patch from the movements of the whisker with the smaller, less distant label. However, such effects were not observed. Whiskers with larger, and more eccentric labels moved in perfect coordination with whiskers with small (routinely used) labels or with unlabeled whiskers. Such observations were made both in mice ($n = 10$ double-labeling experiments) and rats ($n = 10$ double-labeling experiments). We noted no systematic difference in whisker movements as a function of foil patch size from one recording session to the next. These results do not rule out the possibility that foil labels affect whisker movement. If so, however, the animals compensate these effects such that no systematic deviation is apparent.

Whisker movements were videotaped in a small cardboard box, with which the animals had never been in contact before the recording session. Mice and rats spontaneously initiated whisking under such conditions. In guinea pigs, however, it was very difficult to evoke intense whisker movements, and these animals had to be continuously presented with a large number of novel objects in the recording box. We labeled exclusively the laterally oriented C- and D-row whiskers. Only those whisking episodes were analyzed in which the animals kept their head upright above the ground performing no or only small translatory movements. Under these conditions, the foil-labeled whiskers moved in the plane of the video camera, and a small tilt (10°) of the whisker against the lateral plane induced only small errors ($\ll 5\%$) in angular position estimates.

Whisker movements were analyzed offline. Movies were digitized using a framegrabber card and the Image program (Scion, Frederick, MD). Digitized movies were analyzed frame by frame by manually placing cursors on the position of the foil label and the whisker origin. Similarly, we determined the orientation of the animal's head by placing cursors on two landmarks on the animal's head. Both the head orientation values (which usually changed on a much slower time scale than whisker position values) and angular whisker position values were read out from the Scion program into a table/text file. In a second analysis step, these values were imported into a spread sheet program (Excel) and then used to compute for each foil patch position the angular whisker position relative to the rostrocaudal axis of the animal's head. Translatory movements were recognized by tracking landmarks (like the eye) on the animal's head and were corrected for by adjusting the position of the whisker origin. To determine the frequency content of movements, we generated power spectra by computing Fourier transforms of movement traces.

Reverse transcription-PCR. RNA was isolated from mystacial vibrissa of animals as described by Chomczynski and Sacchi (1987). First-strand cDNA was synthesized from 5 μ g of total RNA using reverse transcriptase (Invitrogen, Karlsruhe, Germany) and 1 μ l of pd(N)₆ random hexamer (Amersham Pharmacia, Freiburg, Germany). Oligonucleotides were synthesized in the 3' untranslated regions of four MyHC genes, as described by Sartorius et al. (1998): MyHC1 (sense, 5'-CCAAGGGCCCT-GAATGAGGAG-3'; antisense, 5'-GCAAAGGCTCCAGGTCTGAG-3'), MYHC2A (sense, 5'-AAGCGAAGAGTAAGGCTGTC-3'; antisense, 5'-GTGATTGCTTGC AAAAGGAA C-3'), MyHC2B (sense, 5'-ACAAGCT-GCGGTGAAGAGC-3'; antisense, 5'-CAGGA CAGTGACAAAGA-ACG-3'), MyHC2D (sense, 5'-CCAAGTGCAGGAAAGTGACC-3'; antisense, 5'-AGGAAGAGACTGACGAGCTC-3'). The primers were identical for mouse and rat. For guinea pig, only the MyHC2B primers were available for transcript analysis.

PCR was done with Hotstar Taq polymerase (Qiagen, Hilden, Germany) as: denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, primer extension at 72°C for 30 sec for a total of 30 cycles, followed by 1 cycle of primer extension at 72°C for 10 min. Cycle numbers of PCR were

optimized to yield a linear increase in reaction product. PCR products were visualized directly in ethidium bromide-stained agarose gels. Fluorescence intensities were quantified using IPLab Gel program (Scanalytics, Fairfax, VA).

Myofibrillar ATPase staining. Soleus muscles, EDL muscles, and whole mystacial pads were dissected from animals and embedded in an embedding medium (Sakura, AT Zoeterwoude, The Netherlands). Embedded muscles were frozen rapidly in liquid nitrogen-cooled isopentane and placed on a cryostat at -20°C . Thereafter, $10\ \mu\text{m}$ longitudinal sections in the mid-portion of the muscles were cut and mounted on polylysine-coated glass slides.

Myofibrillar ATPase staining was performed as described by Brooke and Kaiser (1970). In brief, sections were incubated in acidic buffer (3.5 mM barbital and 3.5 mM sodium acetate, pH 4.6) for 15 min and then in ATP staining buffer (3.6 mM ATP, 20 mM barbital, and 18 mM CaCl_2 , pH 9.4) for 2 hr. Incubation in 1% CaCl_2 for 10 min was followed by incubation in 2% CoCl_2 for 10 min and in 2% $(\text{NH}_4)_2\text{S}$ for 1 min. Then sections were dehydrated in 50, 70, 80, 95, and 100% ethanol and mounted on Eukitt (O. Kindler, Freiburg, Germany). Dark-stained fibers (slow fibers) indicate the presence of MyHC1. Fast fibers are recognized as light, unstained fibers containing MyHC2A or intermediate-staining fibers containing MyHC2B or MyHC2D (which represent the fastest fibers). Because the absolute gray values of ATPase stains may vary from trial to trial, we always stained muscles (EDL or soleus) with a roughly known fiber composition together with whisker muscles for internal reference.

Immunocytochemistry. Cross-sections ($10\ \mu\text{m}$) were stained with hematoxylin/eosin to visualize and count muscle fibers. Adjacent sections were used to identify different MyHC forms by isoform-specific antibodies. The sections on glass slides were marked with a PAP pen (G. Kisker, Steinfurt, Germany) and washed briefly in $1\times$ PBS for 5 min. After incubation in blocking solution (BS; 0.5% BSA, 2.5% horse serum, and 0.01% Triton X-100 in PBS) for 30 min, sections were incubated for 2 hr with primary antibody in BS. Sections were then washed three times for 2 min each in PBS and for 10 min in BS. The secondary antibody in BS was applied for 1 hr, and sections were washed four times for 3 min in PBS. Stained sections were visualized under an Axioplan2 fluorescence microscope (Zeiss, Göttingen, Germany) and imaged using a CCD camera (Intas, Göttingen, Germany).

Primary antibody MY-32 (Sigma, Deisenhofen, Germany) was used to detect MyHC1, NOQ7.5.4.D (Sigma) was used to detect all MyHC2 fibers, and A4.74 (Alexis, Grünberg, Germany) was used to detect MyHC2A (Hughes et al., 1993). Anti-MyHC1, MyHC2, and MyHC2A were used at a dilution of 1:1000, 1:200, and 1:10, respectively.

Secondary antibody Alexa 488-labeled goat anti-mouse (Molecular Probes, Leiden, The Netherlands) was used at a dilution of 1:500.

Fiber counts of hematoxylin/eosin, ATPase, and immunocytochemically stained serial sections. Alternating serial sections were stained with hematoxylin/eosin, ATPase staining (as described above), and anti-MyHC antibodies as described above. Images of stained cross-sections were captured with a CCD camera. In soleus ($n = 36$ muscles in mice, $n = 10$ muscles in rats, $n = 4$ muscles in guinea pigs) and EDL ($n = 14$ muscles in mice, $n = 10$ muscles in rats, $n = 4$ muscles in guinea pigs) muscles, the relative number of fiber types could easily be scored based on ATPase-stained sections, by judging the relative intensity of the precipitate stain. Intrinsic whisker muscles (from $n = 8$ whisker pads in mice, from $n = 6$ whisker pads in rats, from $n = 4$ whisker pads in guinea pigs) were considerably more difficult to score because of their homogeneous appearance in the ATPase stain and the difficulty in delineating the outlines of these very small muscle fibers. Thus, intrinsic whisker muscles were scored based on multiple stains using the hematoxylin/eosin stain for delineating individual fibers and relying on both the ATPase staining and the immunocytochemical staining for determining the fiber type.

Results

Whisker movements in mice, rats and guinea pigs: qualitative and quantitative comparisons of movements

In all three species, whisker movements were observed. Novel environments and interactions with novel objects triggered the

most intense movements, suggesting a function of whisker movements in exploratory behavior in all three species. Large whisker movements ($>40^{\circ}$) were common in mice and rats, whereas they were rare but not absent in guinea pigs. In mice and rats, large whisker movements often occurred in bouts of movements (whisking), whereas in guinea pigs large whisker movements often occurred in isolation. Bouts of movements in mice and rats consisted of a rhythmic series of several protractions and retractions. Within a bout of movements, protraction/retraction amplitudes rose and fell continuously. The length of such a whisking bout varied considerably, and a single bout consisted of 4 to >20 protractions and retractions. Under our experimental conditions, most whisking bouts were brief (5–10 protractions and retractions) and were shorter than 1 sec in mice and shorter than 2 sec in rats. Thus, in our behavioral settings, whisking both in rats and in mice was restricted to brief episodes. However, when rats approach or search for their home cage, whisking can occur for extended periods of tens of seconds (K. Ganguly and D. Kleinfeld, personal communication).

All three species kept their whiskers either in a backward, nonerected resting position or the whiskers were moving. Whereas animals can keep the whiskers protracted (for example, when they cross gaps), they always do so only for brief periods. In this respect, whisker movements differ from limb movements in which one observes that a variety of postures can be assumed for prolonged periods.

To provide a better impression of the quantitative differences in whisker movements, we show episodes of particularly intense (i.e., sustained high amplitude) whisker movements for each species in Figure 1. Such episodes were common and relatively stereotyped in mice (Fig. 1A) and rats (Fig. 1B). However, intense whisker movements were rare in guinea pigs. The trace displayed in Figure 1C shows the most intense bout of movements observed (3 hr; two animals). Although whisking frequency was relatively stable, angular amplitudes and velocities of whisking varied widely with the behavioral setting but were generally largest in mice (Fig. 1A, middle) and smallest in guinea pigs (Fig. 1C, middle).

Table 1 gives quantitative values of movement parameters as they were observed for intense whisker movements in mice, rats, and guinea pigs. Whisking in mice occurred with frequencies between 14 and 24 Hz in mice (Table 1; Fig. 1A, right) and between 5 and 12 Hz in rats (Table 1; Fig. 1B, right). In guinea pigs, large whisker movements often occurred in isolation. Bouts of movements were less rhythmic than in rats or mice, and whisking frequencies were ≤ 5 Hz (Table 1; Fig. 1C, right). The rhythmicity of mouse and rat whisker movements led to sharp peaks in the power spectra of the movement recordings (Fig. 1, right). Peaks were even sharper when the analysis was restricted to a single bout of movements (Berg and Kleinfeld, 2003) instead of analyzing of an episode, which contained several whisking bouts and in which movement amplitudes increased and decreased multiple times. Angular movement amplitudes were comparable in mice and rats but were smaller in guinea pigs. Velocities of protraction and retraction were highest in mice, smaller in rats, and smallest in guinea pigs (Table 1), whereby the species differences were more pronounced for average angular velocities than for peak velocities.

Anatomy of the mystacial pads and identification of intrinsic whisker muscles

Figure 2A represents a schematic view of the mouse mystacial pad. Intrinsic whisker muscles form slings surrounding whisker

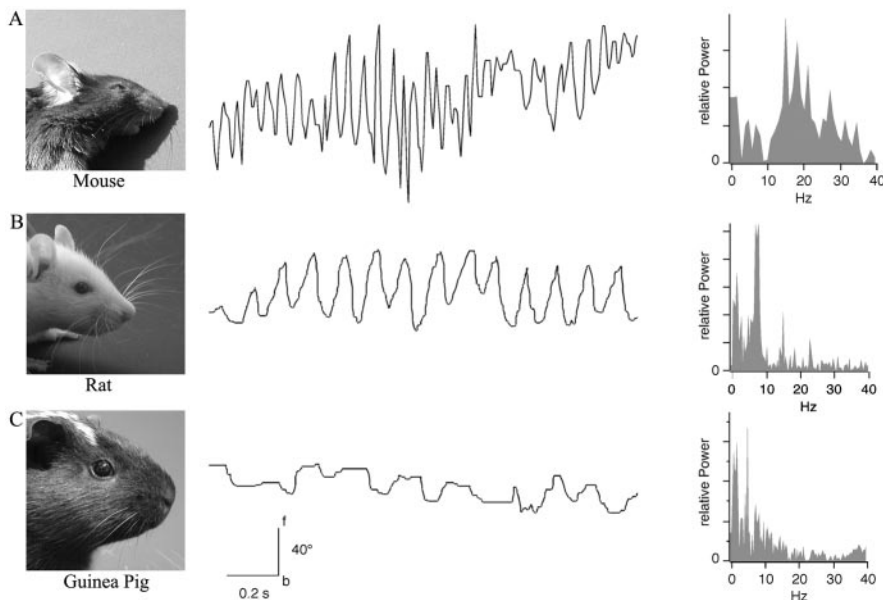


Figure 1. Whisker movements in mouse, rat, and guinea pig. *A*, Left, Side view of a mouse head and whiskers. Middle, Back (b) and forth (f) movements (angular positions) of whisker C2 of a mouse during a whisking episode. Right, Power spectrum of the movement trace. *B*, Left, Rat head. Middle, Rat whisker movements of whisker C2 during a whisking episode. Right, Power spectrum. Conventions are as in *A*. *C*, Left, Guinea pig head. Middle, Guinea pig whisker movements of whisker D2 during an episode of intense whisker movements. Right, Power spectrum. Conventions are as in *A*. All power spectra have been normalized to the same maximum peak height.

Table 1. Movement parameters during episodes of intense whisker movements

	Mouse	Rat	Guinea pig
Whisking frequency (Hz)	20.4 ± 2.8	8.4 ± 1.5	4.7 ± 1.1
Mean angular amplitude during whisking (°)	55 ± 17	56 ± 14	25 ± 11
Average angular velocity for full sweeps of protraction (°/sec)	2074 ± 588	717 ± 186	332 ± 76
Average angular velocity for full sweeps of retraction (°/sec)	2436 ± 791	1484 ± 752	553 ± 276
Maximum angular velocity for full sweeps of protraction (°/sec)	3105 ± 1429	956 ± 385	447 ± 202
Maximum angular velocity for full sweeps of retraction (°/sec)	3700 ± 2194	2022 ± 913	803 ± 187
Peak angular velocity measured during protraction (°/sec) ^a	5925	1950	1350
Peak angular velocity measured during retraction (°/sec) ^a	9900	3500	1800

Values are given as means ± SD. Data refer to 20 episodes of whisking in mice and rats and 5 episodes of intense whisker movements in guinea pigs.

^aValues refer to the maximum value measured across the complete observation episode.

follicles (i; Fig. 2*A,C*), and extrinsic whisker muscles are anchored to the skin (e; Fig. 2*A*.) The attachment of intrinsic and extrinsic muscles to the whisker allows protraction and retraction, respectively (Fig. 2*A*). We prepared 10 μm longitudinal sections of mystacial pads perpendicular to the skin and orthogonal to the orientation of whisker rows, as shown in Figure 2*A*. In hematoxylin/eosin-stained sections, muscle fibers can be recognized by a dark red/violet color, and Figure 2*B* shows three follicles surrounded by a layer of thin intrinsic whisker muscle fibers. Hematoxylin/eosin-stained sections through whole whisker pads indicate that, by far, most of the muscle fibers in the whisker pad belong to the intrinsic whisker muscles. This was true for all three

species studied. Figure 2*C* shows a magnified view of a whisker follicle and the surrounding intrinsic whisker muscle fibers. Figure 2*C* also demonstrates that intrinsic whisker muscle can be identified easily by its characteristic arrangement around the follicle.

MyHC isoforms mRNA expression in extracts from whole mystacial pads

Extracts of whole mystacial pads were made to determine mRNA expression of MyHC isoforms in mystacial pads from mouse, rat, and guinea pig by reverse transcription-PCR (RT-PCR). Because these extracts contain intrinsic and extrinsic whisker muscles and possibly small amounts of other muscle tissue, it is not possible to precisely determine the fiber composition of whisker muscles. Nevertheless, gross MyHC isoform mRNA levels can be determined with molecular resolution that cannot be achieved by ATPase staining or immunochemistry.

Results are summarized in Table 2, revealing in mice predominant expression of MyHC2B mRNA and low levels of MyHC2D mRNA. In rats, we detected only MyHC2B transcripts and none of the

other isoform transcripts tested. In guinea pigs, no appropriate primers for MyHC1, MyHC2A, and MyHC2D mRNAs were available, and we therefore cannot comment on the relative expression of MyHC isoforms in guinea pigs. However, we detected expression of the MyHC2B mRNA.

Comparison of muscle fiber types in soleus, EDL, and intrinsic whisker muscle

We performed myofibrillar ATPase staining, as described by Brooke and Kaiser (1970), to determine the fiber types of intrinsic whisker muscle and various skeletal muscles. Figure 3 shows cross-sections of soleus, EDL, and intrinsic whisker muscles of mouse, rat, and guinea pig after ATPase staining at pH 4.6.

In mice, the intrinsic whisker muscles (Fig. 3*A*) were composed of type 2B or 2D only. The soleus muscle (Fig. 3*B*) contained mainly type 1 and type 2A fibers, with a few type 2B or type 2D (intermediate) fibers, whereas the EDL muscle (Fig. 3*C*) was predominantly composed of type 2A and 2B or 2D fibers, with only a few type 1 fibers.

In rats, the intrinsic whisker muscles (Fig. 3*D*) were predominantly composed of type 2B or 2D with a small number of type 1 fibers. The soleus muscle (Fig. 3*E*) contained mainly type 1 fibers, with a small number of type 2A and a few type 2B or type 2D fibers, whereas the EDL muscle (Fig. 3*F*) predominantly comprised type 2A and type 2B or 2D fibers, with smaller number of type 1 fibers.

In guinea pigs, the intrinsic whisker muscles (Fig. 3*G*) contained type 2B or type 2D fibers, with small number of type 2A and a few type 1 fibers. The soleus muscle (Fig. 3*H*) only contained type 1 fibers, whereas the EDL muscle (Fig. 3*I*) was predominantly composed of type 2A and type 2B or 2D fibers, with a few type 1 fibers.

It should be kept in mind that myofibrillar ATPase staining does not differentiate between MyHC2B- or MyHC2D-containing fibers (both fiber types display intermediate staining).

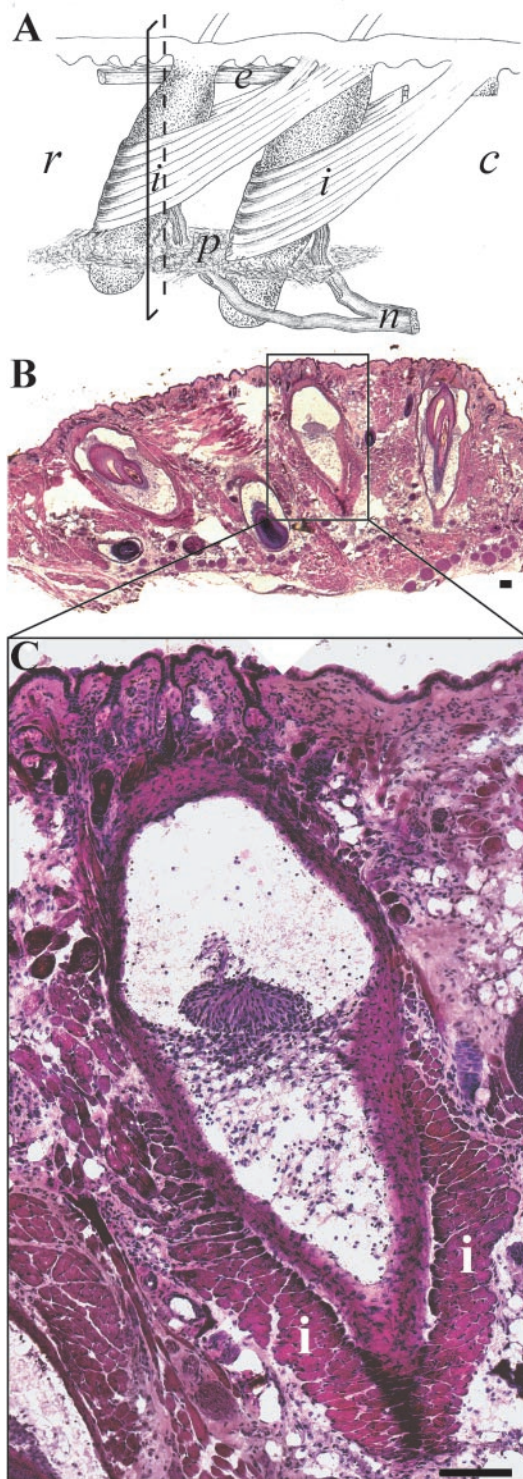


Figure 2. Cross-section of a mouse mystacial pad. *A*, Schematic overview of vibrissa follicles [after Dörrfl (1982)]. *r*, Rostral; *c*, caudal; *n*, motor nerve; *p*, plate; *i*, intrinsic whisker muscle; *e*, extrinsic whisker muscle; rectangle marks longitudinal section across mystacial pad as shown in *B* and *C*. *B*, Mystacial pad stained with hematoxylin/eosin. *C*, Magnified view from *B* as outlined by rectangle. *i*, Intrinsic whisker muscles. Scale bar, 100 μ m.

Also, the intrinsic whisker muscle fibers are smaller than soleus and EDL muscles, and the boundaries and shape of the single fibers were not outlined clearly in each case. Therefore, it is not possible to characterize fiber types of intrinsic whisker muscle as exactly as for soleus or EDL muscle using the myofibrillar ATPase staining.

Table 2. MyHC isoform mRNAs in mystacial pads

	Proportion of MyHC isoform mRNAs (%)			
	MyHC1	MyHC2A	MyHC2B	MyHC2D
Mouse	nd	nd	89.5 \pm 3.54	10.5 \pm 3.54
Rat	nd	nd	100	nd
Guinea pig	?	?	+	?

Total RNA was extracted from whole mystacial pads of mouse, rat, and guinea pig and analyzed by RT-PCR. The fluorescence intensities of the ethidium bromide-stained PCR products as observed on agarose gel electrophoresis were used to compare the MyHC isoform mRNA levels in different species. nd, Not detected; ?, no appropriate primers for these mRNAs were available.

Immunochemical staining of MyHC isoforms in the whisker pad and muscle fiber counts

To ascertain the exact distribution of fiber types in the intrinsic whisker muscle of all three species, we used isoform-specific anti-MyHC antibodies. Because it is difficult to exactly delineate the shape of each fiber after immunochemical staining, we prepared serial sections and performed hematoxylin/eosin staining and immunochemical staining in alternating sections.

Figure 4 shows the distribution of fibers in intrinsic whisker muscle as revealed by antibody labeling. The hematoxylin/eosin staining was used to visualize the morphology of intrinsic whisker muscles and to count the total number of fibers per muscle section (Fig. 4*A, E, I*). The fibers of intrinsic whisker muscles from mouse (Fig. 4*C*) reacted exclusively with anti-MyHC2 antibodies, and there were no fibers stained by anti-MyHC1 (Fig. 4*B*) or anti-MyHC2A (Fig. 4*D*) antibodies. Intrinsic whisker muscles of the rat (Fig. 4*G*) were stained by anti-MyHC2 antibodies and, in a few cases, by anti-MyHC1 antibodies (Fig. 4*F*), but there was no labeling by anti-MyHC2A antibodies (Fig. 4*H*). Intrinsic whisker muscles of guinea pigs (Fig. 4*K*) also reacted predominantly with anti-MyHC2. Some fibers were labeled by anti-MyHC2A (Fig. 4*L*) and infrequently also by anti-MyHC1 (Fig. 4*J*) antibodies.

The MyHC-typing of muscle fibers, together with the data from myofibrillar ATPase staining, are shown in Figure 5. As explained in Materials and Methods, MyHC-typing of muscle fibers in soleus and EDL was done by ATPase staining, whereas MyHC-typing of muscle fibers in the intrinsic whisker muscles was based on serial sections stained by hematoxylin/eosin, ATPase staining, and antibodies. Because both myofibrillar ATPase staining and immunochemical staining cannot distinguish MyHC2B- and MyHC2D-containing fibers, we refer to these fibers as MyHC2B/2D fibers here and in Fig. 5.

Intrinsic whisker muscles of mice were composed exclusively of type MyHC2B/2D fibers, and mRNA expression suggests that the predominant fraction of intrinsic whisker muscle fibers was of type 2B ($\geq 90\%$). Intrinsic whisker muscles of rats were composed of fiber type MyHC1 ($10 \pm 1\%$) and MyHC2B/2D ($90 \pm 1\%$). Because no MyHC2D mRNA was detectable in these muscles, the data suggest that $\sim 90\%$ of rat intrinsic whisker muscles fibers were of type 2B. Intrinsic whisker muscles of guinea pigs were composed of fiber type MyHC1 ($3 \pm 1\%$), MyHC2A ($19 \pm 1\%$), and MyHC2B/2D ($78 \pm 1\%$).

The MyHC-typing of muscle fibers of skeletal muscles led to strikingly different results (Fig. 5). Intrinsic whisker muscles all three species are characterized by their high content of type MyHC2B/2D fibers and differ from skeletal muscles even when comparing them to the EDL, a muscle specialized for fast movements.

Fiber composition of extrinsic whisker muscles

In our study, we focused on intrinsic whisker muscles because they form the bulk of the whisker musculature and are easily

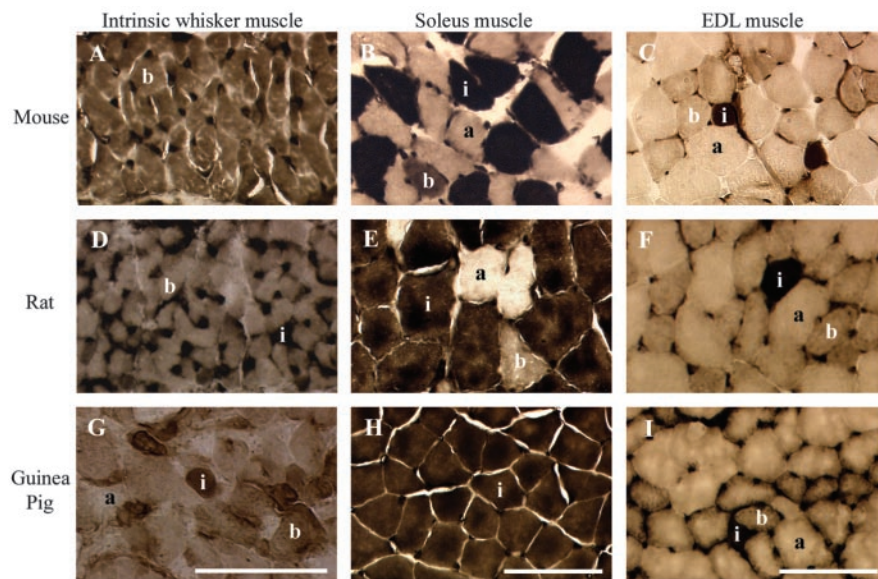


Figure 3. Serial cross-sections of different muscle types from mouse, rat, and guinea pig. Cross-sections of intrinsic whisker, soleus, and EDL muscles were subjected to myofibrillar ATPase staining at pH 4.6: mouse (A–C), rat (D–F), and guinea pig (G–I). Intrinsic whisker muscle of mystacial pads (A, D, G), soleus muscle (B, E, H), and EDL muscle (C, F, I). i, Muscle fiber type 1; a, muscle fiber type 2A; b, muscle fiber type 2B or 2D. Scale bars, 50 μ m; applies to all columns.

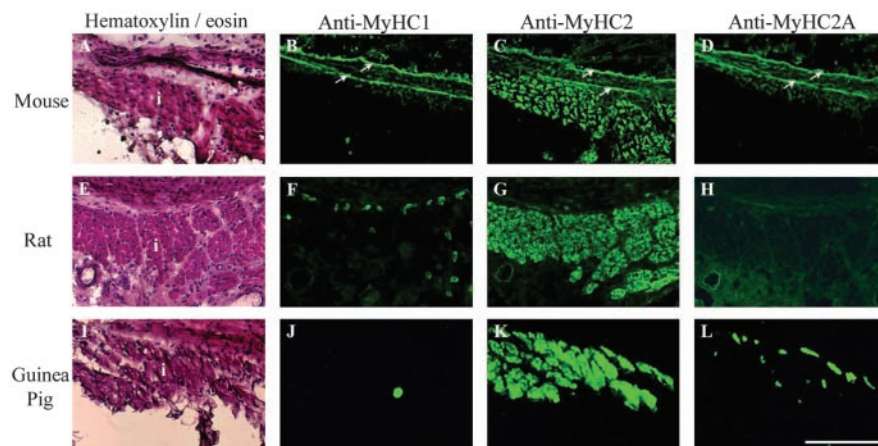


Figure 4. Immunohistochemical characterization of intrinsic whisker muscles. Serial cross-sections of intrinsic whisker muscles from mouse (A–D), rat (E–H), and guinea pig (I–L) were stained with specific MyHC isoforms. A, E, I, Hematoxylin/eosin staining of adjacent sections were done to visualize individual muscle fibers. MY-32 antibodies were used to detect MyHC1-containing fibers (B, F, J), NOQ7.5.4.D antibodies were used to detect MyHC2-containing fibers (C, G, K), and A4.74 antibodies were used to detect MyHC2A-containing fibers (D, H, L). Anti-MyHC1, MyHC2, and MyHC2A were used at a dilution of 1:1000, 1:200, and 1:10, respectively. Arrows indicate the wall of the vibrissal capsule, which in mice shows an unspecific (nonmuscular) antibody signal. i, Intrinsic whisker muscle. Scale bar, 100 μ m.

identified by their characteristic arrangement around the follicle. Extrinsic whisker muscles run as thin bundles below the skin and are less easily identified (Dörfl, 1982; Wineski, 1985). Occasionally, we detected thin round bundles of muscle fibers below the skin that could be followed through multiple sections. Preliminary evidence suggests that, in the species investigated here, these putative extrinsic whisker muscles were also predominantly composed of fast type 2B fibers: (1) such bundles were stained specifically by anti-MyHC2 antibodies (data not shown); (2) ATPase yielded intermediately stained fibers, the staining of which was comparable with the staining of type 2B fibers of the intrinsic whisker muscle; and (3) the mRNA expression suggests the predominant expression of MyHC2B transcripts. Because of the

small sample of muscles studied here, we could not determine the exact distribution and MyHC composition of muscle fibers of extrinsic whisker muscles.

Discussion

Mice, rats, and guinea pigs can generate fast and large amplitude whisker movements. The main species difference in whisker movements consists of the fact that guinea pigs do not show bouts of fast, strictly rhythmic whisker protractions and retractions (i.e., whisking). The average speed of whisker movements is much lower in guinea pigs than in mice and rats. In all three species, intrinsic whisker muscles are composed predominantly of fast type 2B/2D fibers. In mice, type 2B/2D fibers seem to constitute the only fiber type, whereas in rats we also observed \sim 10% of slow type 1 fibers, and in guinea pigs we observed \sim 3% slow type 1 fibers and 20% of fast type 2A fibers. The MyHC transcript analysis suggests that \geq 90% of the type 2B/2D fibers in rats and mice actually correspond to type 2B fibers. The combination of methods used here allowed us to determine the fiber type composition of intrinsic whisker muscle with a high degree of certainty. The MyHC transcript analysis by RT-PCR determines with high specificity the expression levels of individual MyHC mRNAs, whereas ATPase staining of MyHC isoforms as well as antibody staining extends this analysis on the protein level and allows us to classify muscles according to their content of different MyHC isoforms. This information will help us to understand how muscle function is regulated.

Type 2B fibers predominate in whisking musculature

Our results on whisker movements are in agreement with previous studies on the whisking behavior of rats (Welker, 1964; Carvell and Simons, 1990; Harvey et al., 2001; Sachdev et al., 2002; Berg and Kleinfeld, 2003), mice (Lee and Woolsey, 1975), and informal observations on guinea pigs (Woolsey et al., 1975). Our observations

on the anatomy of the whisker pad are consistent with the findings from mice (Dörfl, 1982) and hamsters (Wineski, 1985).

Very little work has been done on the muscle fiber composition of the whisker muscles. Wineski (1985), after pioneering work on the classification of whisker muscles, published a number of preliminary results on fiber types of whisker muscles. These studies on hamsters, rats, and guinea pigs (Wineski et al., 1992; Wineski and Pitts, 1994) were based on ATPase stains and also described a majority of fast type 2 fibers in the whisker pad. In contrast with our results, these studies reported a predominance of type 2A fibers in the whisker pad (Wineski et al., 1992;

Wineski and Pitts, 1994). However, it must be emphasized that type 2A and 2B fibers can be difficult to distinguish in ATPase staining. Our additional analyses based on mRNA levels and antibody labeling suggests that, in fact, 2B and not 2A fibers are the dominant fiber type of the whisker musculature.

In most respects, our findings on the fiber composition of soleus and EDL muscles confirm previous findings (Hughes et al., 1999; Totsuka et al., 2003). However, in our study we observed a lower fraction of 2B fibers in the EDL than previously reported (Hughes et al., 1999); most likely, this difference results from the fact that our sample of fibers of the EDL muscle came from more axial parts of the EDL, which are known to consist of a lower percentage of 2B fibers (Lyons et al., 1983; Narusawa et al., 1987).

Evolutionary aspects

The fiber composition of a muscle reflects the characteristics of the movements the muscle is involved in. The soleus muscle, a muscle mainly generating slow movements, contains predominantly type 1 and type 2A fibers, with small proportions of type 2B fibers, whereas the EDL muscle, a fast muscle, predominantly contains type 2B fibers, with a smaller portion of type 2A fibers (Schmalbruch, 1985).

Comparison of the fiber composition of these muscles with intrinsic whisker muscle shows that whisker movements are generated by a musculature that is unique in its large fraction of type 2B fibers. Type 2B fibers provide the highest twitch velocity (Bottinelli, 1991; Schiaffino and Reggiani, 1996). High contraction speed does not come for free, however, and is associated with high rates of ATPase activity (Barany, 1967). Thus, our observations suggest that the need for high contraction speed may have been a driving force in the evolution of whisker muscles. This idea is also consistent with the fact that mice, which perform the fastest whisker movements of the three species studied, have exclusively type 2B fibers and that rats, which perform faster whisker movements than guinea pigs, have a higher percentage of type 2B fibers than guinea pigs.

Another feature that distinguishes type 2B fibers from other muscle fibers is their high susceptibility to fatigue. The fact that under most conditions both rats and mice whisk only for brief periods and that the whiskers rarely assume a constant protracted position might be related to this lack of fatigue resistance. However, under certain conditions at least, rats seem to be able to whisk for extended periods (Ganguly and Kleinfeld, personal communication), making this idea less compelling; it would be worth investigating whether animals meet their metabolic limits after such extended episodes of whisking. For skeletal muscles involved in the locomotion of predators, it has been observed that sedentary predators like cats and lions that engage in sprints have a large fraction of type 2B fibers (Armstrong et al., 1977). In contrast, the more continuously active dogs lack type 2B fibers (Armstrong et al., 1982; Snow et al., 1982). In analogy, one might argue that whisking occurs in a more "sprint-like" than continuous manner, although it is not strictly limited to very brief episodes. Finally, it has been suggested that type 2B fibers might be advantageous because of their low-energy demands during periods of inactivity (Burke, 1994), but proof for this idea is pending.

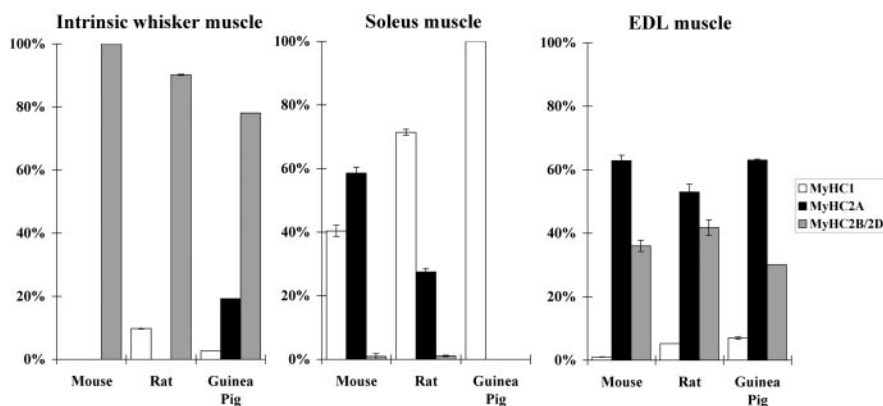


Figure 5. Fiber type composition of intrinsic whisker, soleus, and EDL muscles. Serial cross-sections of muscles were stained with hematoxylin/eosin, myofibrillar ATPase staining, and with antibodies specific to MyHC isoforms. Stained muscle sections were imaged using a CCD camera, and the number of the various fiber types was counted manually.

It seems to be the rule rather than the exception that muscles involved in generating movements of sensory organs are specialized for high-speed contractions. Thus, it was shown that in extraocular muscles of the eye (Wieczorek et al., 1985; Sartore et al., 1987) and in the tensor tympani muscle of the ear (Mascarello et al., 1982) specialized unconventional myosin isoforms are expressed that are thought to mediate very fast contractions.

We conclude that the high-speed movements of whiskers are mediated by a specialized musculature predominated by fast contracting type 2B fibers, and we argue that such a high-speed motor apparatus may have evolved for fast scanning of the sensory environment.

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