Reviewing the Morphology of the Jaw-Closing Musculature in Squirrels, Rats, and Guinea Pigs with Contrast-Enhanced MicroCT

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ABSTRACT

Rodents are defined by their unique masticatory apparatus and are frequently separated into three nonmonophyletic groups—sciuromorphs, hystricomorphs, and myomorphs—based on the morphology of their masticatory muscles. Despite several comprehensive dissections in previous work, inconsistencies persist as to the exact morphology of the rodent jaw-closing musculature, particularly, the masseter. Here, we review the literature and document for the first time the muscle architecture noninvasively and in 3D by using iodine-enhanced microCT. Observations and measurements were recorded with reference to images of three individuals, each belonging to one of the three muscle morphotypes (squirrel, guinea pig, and rat). Results revealed an enlarged superficial masseter muscle in the guinea pig compared with the rat and squirrel, but a reduced deep masseter (possibly indicating reduced efficiency at the incisors). The deep masseter had expanded forward to take an origin on the rostrum and was also separated into anterior and posterior parts in the rat and squirrel. The zygomaticomandibularis muscle was split into anterior and posterior parts in all the three specimens by the masseteric nerve, and in the rat and guinea pig had an additional rostral expansion through the infraorbital foramen. The temporalis muscle was found to be considerably larger in the rat, and its separation into anterior and posterior parts was only evident in the rat and squirrel. The pterygoid muscles were broadly similar in all three specimens, although the internal pterygoid was somewhat enlarged in the guinea pig implying greater lateral movement of the mandible during chewing in this species. Anat Rec, 294:915–928, 2011. © 2011 Wiley-Liss, Inc.

Key words: microCT; contrast-enhanced; rodent; masseter; temporalis; pterygoid

The masticatory apparatus of rodents is unique among mammals and defines the order Rodentia. As well as possessing an extremely unusual dentition—a pair of grossly enlarged incisors separated from the cheek teeth by a large diastema—rodents have a highly distinctive arrangement of masticatory muscles. The masseter is by far the dominant jaw-closing muscle in the Rodentia, comprising between 60% and 80% of the entire masticatory muscle mass (Turnbull, 1970). Furthermore, the musculature has become specialized to accomplish not only gnawing at the incisors and chewing at the molars

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Aplodontia rufa is one of the most pre-Oligocene fossil rodents and also in the extant species. The primitive arrangement of rodent masticatory systems has persisted, largely thanks to Wood (1965). In his work, Wood describes the arrangement of jaw-closing muscles in particular, the masseter, has long been used to classify the rodents into subgroups. Brandt (1855) first used features primarily from the masticatory apparatus to group rodents into squirrel-like (Sciuromorpha), mouse-like (Myomorpha), and porcupine-like (Hystricomorpha) forms (Brandt's fourth group). The rabbits, hares, and pikas, now occupy a separate, albeit closely related, order. These three suborders were largely retained with only minor revisions by most workers for the next century (e.g., Thomas, 1896; Miller and Gidley, 1918) and indeed were still the basis for rodent taxonomy in George Gaylord Simpson's monumental classification of the mammals in 1945. It should be noted, however, that Simpson alluded to a growing dissatisfaction with the three suborders (Simpson, 1945, p.198) but retained them in his work owing to a lack of a better alternative at that time. The problem with the three suborder arrangement can be clearly seen in Simpson's classification: there are a number of rodent families that do not neatly fit into the Sciuromorpha, Myomorpha, or Hystricomorpha. In particular, the Anomaluridae (scaly-tailed squirrels), Pedetidae (springhares), Dipodidae (jerboas, jumping mice, and birchmice), Bathyergidae (mole-rats), and Ctenodactylidae (gundis) have all posed problems to various workers in the past. A competing classification of rodents, first proposed by Tullberg (1899), split the Rodentia into two suborders (Sciurognathi and Hystricognathi) based on the morphology of the angular process of the mandible. This system overlaps with the masseter-based classification in some respects, such as the fact that all hystricognaths have a hystroicormus muscle arrangement (Lavocat, 1974; Wood, 1974), but has notable differences as well, particularly that sciurognaths can possess any of the three masticatory muscle morphologies (Offermans and De Vree, 1989).

Neither of the two classifications outlined above has stood the test of time. Although evidence points toward a monophyletic Hystricognathi, the Sciurognathi is almost certainly a paraphyletic grouping, and the idea that the three suborders of Brandt (1855) and Simpson (1945) represent monophyletic groups of rodents is now generally discredited (Adkins et al., 2001; Huchon et al., 2002; Adkins et al., 2003; Blanga-Kanfi et al., 2009). However, the use of the terms sciurocorm, myomorph, and hystricormus as adjectives describing particular arrangements of jaw-closing muscles has persisted, largely thanks to Wood (1965). In his work, Wood describes the primitive arrangement of rodent masticatory muscles (the “protogomorph” condition, found in most pre-Oligocene fossil rodents and also in the extant mountain beaver, *Aplodontia rufa*), and the three arrangements derived from it. In the sciurocorm condition, part of the masseter has expanded posterodorsally to take its origin from the rostrum and the widened root of thezygomatic arch. This arrangement is seen in the Sciuridae (squirrels), Castoridae (beavers), and Geomyoidea (pocket gophers, kangaroo rats, and mice). In the hystricormuscormian apparatus, a deeper part of the masseter has extended forward, through the orbit and the grossly enlarged infraorbital foramen to take an origin on the snout. This morphology is found in the Caviomorpha (South American rodents), Phiomorpha (African mole-rats, cane rats, and the dassie rat) and Hystricidae (old world porcupines) as well as the previously mentioned Pedetidae, Anomaluridae, Dipodidae, and Ctenodactylidae. Finally, the myomorphs combine sciurocorm and hystricorm morphology features with the origins of both parts of the masseter having migrated on to the rostrum. This condition is seen in the Muroidea (mice and rats) and the Gliridae (dormice).

The above morphological descriptions have been greatly complicated by the complex lack of consensus on the nomenclature of rodent masticatory muscles, with particular regard to the masseter (hence the lack of specific muscle nomenclature in the previous paragraph). Part of the confusion arises due to the uncertainty of how many layers the masseter divides into, and whether all of these layers should be referred to as the masseter or as entirely separate muscles. This uncertainty persists despite several thorough descriptive articles based on gross dissection (in particular, compare Hiiemae and Houston, 1971 with Wejs, 1973; see also Turnbull, 1970; Woods, 1972; Cooper and Schiller 1975; Ball and Roth, 1995; Druzinsky, 2010a). A possible cause of much of this uncertainty is methodological in that information on muscle morphology is typically gathered invasively by dissection, and thus, superficial structures are destroyed in gaining access to the deeper structures and spatial relationships are difficult to define accurately. On the basis of the work by Metscher (2009), Jeffery et al. (2011) recently developed an alternative, nondestructive approach that has the potential to reveal detailed information on rodent masticatory muscle morphology in situ. In this method, the biological specimen is soaked in a solution of iodine for a number of weeks before scanning, which allows the detailed imaging of soft tissues and hard tissues. Here, we use this novel technique in reviewing the structure, arrangement, and terminology of the masticatory apparatus in three rodents displaying the sciurocorm, hystricorm, and myomorph muscle arrangements. Descriptions of the masticatory muscles will be reported, comparisons with previous work will be made, and current controversies will be addressed. Given the importance of rodents and knowledge of their musculature to many scientific disciplines other than anatomy, in particular evolutionary developmental biology (Hallgrímsson et al., 2007; Jones et al., 2007; Hallgrímsson and Lieberman, 2008; Parsons et al., 2008), it is felt that such a review is timely and necessary.

**MATERIALS AND METHODS**

**Sample**

Three rodent species were chosen that possess the sciurocorm, hystricorm, and myomorph morphologies. These were, respectively, the Eastern grey squirrel (*Sciurus carolinensis*), the domesticated guinea pig (*Cavia porcellus*), and the brown rat (*Rattus norvegicus*). These species were selected as they all have been well-studied previously, and each represents a typical member of its feeding type (i.e., none is anomalously...
specialized). To select an average individual of each species to study, formalin-fixed heads of eight rats, eight guinea pigs, and seven squirrels were imaged using microCT. Subsequently, 59 three-dimensional landmarks were taken from the skull and mandible of each specimen using Amira 5.2 (Mercury Systems, Chelmsford, MA). From this data, variation in the shape of the skull and mandible within each species was analyzed using geometric morphometrics (O'Higgins, 2000; Adams et al., 2004), implemented with the software Morphologika² v2.5 (O'Higgins and Jones, 1998). The landmark coordinates were subjected to Procrustes superimposition to remove translation, rotation, and size differences, and then a principal components analysis was performed for each species. For each specimen, the squared value of each PC score was multiplied by the percentage of variation encapsulated by that principal component, and the results were summed over the first seven principal components. The specimen with the lowest total within each species was determined to be the individual closest to the mean shape of the sample (which is located at the origin of the principal axes, O'Higgins, 2000), and this individual was used to study the masticatory musculature.

**Imaging**

To visualize the muscle tissues and the bone in a non-destructive manner, the specimen best approximating the mean shape of the sample of each species was imaged again, this time using contrast-enhanced microCT. The enhancement uses a solution of iodine (I₂KI) to increase the differential attenuation of X-rays among soft tissues and has been shown to demonstrate patterns of muscle fibers and fascicles against the connective tissues (Jeffery et al., 2011). Specimens were fixed in phosphate-buffered formal saline solution (polymerized formaldehyde dissolved as a 4% solution in phosphate-buffered saline allowing for long term storage with limited tissue shrinkage) and then placed in 15 to 25% I₂KI contrast agent for a period of between 2 to 7 weeks depending on size. These incubation times were insufficient for the passive diffusion throughout the larger Cavia specimen. Small volumes of contrast agent (15%) were therefore injected into the body of the muscles with a fine grade needle and incubated for a further 2 weeks. All three specimens were imaged with the Metris X-Tek custom 320 kV bay system at the EPSRC funded Henry Moseley X-ray Imaging Facility, University of Manchester. Imaging parameters were optimized for each specimen to maximize spatial and contrast resolution as well as data handling. Voxel resolutions varied from 0.033 to 0.040 mm.

**Observations and Reconstructions**

The contrast-enhanced microCT images (e.g., Figs. 1–3) were viewed using Amira 5.2. The morphology of each jaw-closing muscle was determined and described. Three-dimensional reconstructions of all the jaw-closing muscles were created for each specimen using the segmentation function of Amira 5.2 (e.g., Fig. 4). Reconstructions of the skull and mandible were also created to facilitate visualization of the origins and insertions of each muscle. As can be seen from the microCT images (e.g., Figs. 1–3), the difference in contrast between muscle and bone was not sufficient to allow the models to be generated using the threshold function. Hence, the muscle and bone reconstructions were built by manually painting the object of interest in a number of slices and interpolating between them. A smoothing function was
used to reduce the blocky appearance of the reconstructions. Amira 5.2 was also able to output the volume of each muscle in each reconstruction. These were converted to masses assuming muscle density of 1.0564 g/cm³ (Murphy and Beardsley, 1974). The condylar skull length for each specimen was measured from the
microCT scans. This length was used to estimate body mass, using the independent contrasts-corrected formula given by Reynolds (2002).

The observed arrangements of the jaw-closing muscles were compared with those seen in previous literature, both of the three species under study and other rodent species. The literature consulted was as follows: Ball and Roth, 1995 (Sciurus, Microsciurus, Sciurillus, Tamiasciurus, Tamias, Glaucymy); Byrd, 1981 (Cavia); Druzinsky, 2010a (Aplodontia, Cynomys, Tamias, Marmota, Rattus, Sciurus, Thryonomys); Greene, 1935 (Rattus); Hiemae and Houston, 1971 (Rattus); Müller, 1933 (Hydrochoerus); Offermans and De Vree, 1989 (Pedetes); Olives et al., 2004 (Aconemys, Octomys, Tymanocottomys, Spalacopus, Octodon, Octodontomy); Satoh, 1997, 1998, 1999 (Apodemus, Cletetula, Tscherkia, Phodo-
pus); Satoh and Iwaku, 2006 (Onychomys); Satoh and Iwaku, 2009 (Neotoma, Peromyscus); Schumacher and Rehmer, 1962 (Cavia, Rattus); Thorlington and Darrow, 1996 (Aplodontia, Paraxerus, Fumiscius, Heliosciurus, Protoxerus, Funambulus, Callioseciurus, Tamiops, Xerus, Atlantoferus, Ratufa); Turnbull, 1970 (Sciurus, Rattus, Hystrix); Weis, 1973 (Rattus); Wood, 1965 (Marmota, Myocastor, Ondatra); Wood and White, 1950 (Chinchilla); Woods, 1972 (Proechimys, Echimys, Isotherix, Mesomyom, Myocastor, Octodon, Ctenomys, Erethizina, Cavia, Chinchilla, Dasyprocta, Thryonomys, Petromus); Woods and Howland, 1979 (Capromys, Geocapromys, Pilagiodontina, Myocastor); Woods and Hermanson, 1985 (Capromys, Geocapromys, Plagiodontina, Myocastor, Echimys, Octodon, Erethizina, Coendou, Dasyprocta, Atherurus, Thryonomys, Petromus).

**Nomenclature**

In this article, the three layers of the masseter will be referred to as the superficial masseter, the deep masseter, and the zygomasticomandibularis (following Turnbull, 1970; Weis, 1973; Ball and Roth, 1995 among others). There are a number of nomenclatures currently in use, all of which have their advantages. The principal competing system, favoured by Wood (1965) and Woods (1972), names the three layers superficial masseter, lateral masseter and medial masseter; but also see Satoh and Iwaku (2004, 2006, 2009) and Druzinsky (2010a,b) who combine different parts of these two nomenclatures. The system used here has been chosen for its consistency with the nomenclature used in most other mammalian groups (e.g. Storch, 1968; Coldiron, 1977; Janis, 1983). The rostral expansion of the innermost layer in myomorphs and hystricomorphs, sometimes known as the maxillomandibularis (e.g. Müller, 1933; Schumacher and Rehmer, 1962; Turnbull, 1970), is here termed the infraorbital part of the zygomaticomandibularis. Where the temporalis has been split into two parts, they are named the medial and lateral parts, as this is felt to reflect more accurately their anatomical relationship to one another than anterior and posterior. The pterygoid muscles are referred to as internal and external in reference to their origin in and on the pterygoid fossa.

**RESULTS**

Table 1 shows the masses of all jaw-closing muscles and the relative proportion of the total muscle mass that each comprises for all three specimens. Table 2 gives the condylodial skull length and estimated body mass of each specimen to allow comparison with other literature. Figures 1–3 demonstrate the enhanced microCT imaging of the muscles in the squirrel, rat, and guinea pig, respectively. Regions of light (high X-ray attenuation) represent groups of muscle fibers (see Jeffery et al., 2011). Darker bands represent the epimysium and perimysium that separate muscles and fascicles. It can be seen that although the microCT images of the guinea pig (Fig. 3) are less well resolved (due in part to the larger size of the specimen) than those of the squirrel and rat (Figs. 1 and 2), the separation between muscle layers can still be distinguished.
TABLE 1. Masses and percentages of jaw-closing muscles

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Squirrel</th>
<th>%</th>
<th>Guinea pig</th>
<th>%</th>
<th>Rat</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial masseter</td>
<td>0.123</td>
<td>27.37</td>
<td>0.219</td>
<td>45.35</td>
<td>0.064</td>
<td>20.42</td>
</tr>
<tr>
<td>Deep masseter</td>
<td>0.162</td>
<td>36.04</td>
<td>0.037</td>
<td>7.57</td>
<td>0.108</td>
<td>34.34</td>
</tr>
<tr>
<td>Anterior</td>
<td>0.099</td>
<td>21.96</td>
<td>0.007</td>
<td>1.40</td>
<td>0.047</td>
<td>14.91</td>
</tr>
<tr>
<td>Posterior</td>
<td>0.063</td>
<td>14.07</td>
<td>0.071</td>
<td>14.75</td>
<td>0.061</td>
<td>19.43</td>
</tr>
<tr>
<td>Zygomaticomandibularis</td>
<td>0.039</td>
<td>8.70</td>
<td>0.071</td>
<td>14.75</td>
<td>0.021</td>
<td>6.76</td>
</tr>
<tr>
<td>Anterior</td>
<td>0.030</td>
<td>7.77</td>
<td>0.029</td>
<td>5.95</td>
<td>0.005</td>
<td>1.59</td>
</tr>
<tr>
<td>Posterior</td>
<td>0.009</td>
<td>1.93</td>
<td>0.006</td>
<td>1.32</td>
<td>0.003</td>
<td>0.94</td>
</tr>
<tr>
<td>Infraorbital</td>
<td></td>
<td></td>
<td>0.090</td>
<td>18.68</td>
<td>0.013</td>
<td>4.24</td>
</tr>
<tr>
<td>Temporalis</td>
<td>0.054</td>
<td>12.03</td>
<td>0.051</td>
<td>10.65</td>
<td>0.085</td>
<td>26.86</td>
</tr>
<tr>
<td>Lateral</td>
<td>0.006</td>
<td>1.40</td>
<td>0.024</td>
<td>7.73</td>
<td>0.060</td>
<td>19.14</td>
</tr>
<tr>
<td>Medial</td>
<td>0.048</td>
<td>10.63</td>
<td>0.060</td>
<td>19.14</td>
<td>0.060</td>
<td>19.14</td>
</tr>
<tr>
<td>Ptterygoid</td>
<td>0.071</td>
<td>15.87</td>
<td>0.105</td>
<td>21.68</td>
<td>0.037</td>
<td>11.62</td>
</tr>
<tr>
<td>External</td>
<td>0.019</td>
<td>4.30</td>
<td>0.014</td>
<td>3.00</td>
<td>0.010</td>
<td>3.33</td>
</tr>
<tr>
<td>Internal</td>
<td>0.052</td>
<td>11.56</td>
<td>0.090</td>
<td>18.68</td>
<td>0.026</td>
<td>8.29</td>
</tr>
<tr>
<td>Total</td>
<td>0.450</td>
<td>100.00</td>
<td>0.483</td>
<td>100.00</td>
<td>0.315</td>
<td>100.00</td>
</tr>
</tbody>
</table>

TABLE 2. Skull length and estimated body mass of rodents in this study

<table>
<thead>
<tr>
<th>Skull length (mm)</th>
<th>Estimated body mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squirrel</td>
<td>48.22</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>57.51</td>
</tr>
<tr>
<td>Rat</td>
<td>43.40</td>
</tr>
</tbody>
</table>

Superficial Masseter

The outermost layer of the masseter is clearly distinguished from the deeper layers in the microCT images. There is a clear dark band separating the superficial masseter from the deep masseter in coronal view in all three rodents (Figs. 1–3). From the reconstructions (Fig. 4), it can be seen that the superficial masseter exhibits a fairly consistent morphology across the three specimens. This muscle takes a small origin from a flattened tendon attached to a small tubercle or process just below the infraorbital foramen. The posterodorsal muscle fascicles then run to the back of the mandible to insert into a small region on the posterolateral surface of the angle of the jaw ramus. Some of the muscle fascicles on the dorsal edge of this muscle also insert on to the aponeurosis of the deeper masseteric layer, making the separation of these two parts of the masseter frequently difficult. The anteroverentral fascicles of the superficial masseter run under the mandible to insert on the medial surface of the jaw in a fossa just ventral to the insertion of the internal pterygoid (Figs. 1–3). In the rat, there is also a dorsal elongation of this reflected part of the superficial masseter anterior to the internal pterygoid (Fig. 2). This pars reflexa (Turnbull, 1970; Woods, 1972; Weijts, 1973) is even more well-developed in the guinea pig, extending as far as the medial condyloid process (Fig. 3). It can be seen from Table 1 that the superficial masseter is relatively larger in the guinea pig (Fig. 4a) than in the rat or squirrel (Fig. 4b,c), forming almost half of the jaw-closing musculature. Its dorsal edge is at the level of the zygomatic arch, almost completely obscuring the deeper layers in lateral view. In contrast, the superficial masseter of the rat and squirrel is much more restricted dorsally, its margin running diagonally from the origin at the front of the zygomatic arch to the tip of the angle, thus revealing the deep masseter behind.

Some workers have divided this muscle into two parts based on the two insertion areas on the lateral and medial surfaces of the mandible (Greene, 1935; Yoshikawa and Suzuki, 1969; Woods, 1972; Woods and Howland, 1979; Woods and Hermanson, 1985). However, this study, along with many others (e.g., Wood, 1965; Weijs, 1973; Ball and Roth, 1995; Thorington and Darrow, 1996; Druzinsky, 2010a), retains it as a single muscle mass as there is no clear separation seen in the contrast-enhanced microCT images.

Deep Masseter

This muscle layer, sandwiched between the superficial masseter and zygomaticomandibularis (Fig. 5), takes its origin from the ventrolateral surface of the zygomatic arch and inserts on the lateral surface of the mandible all along the masseteric ridge from beneath the second molar to the angular process. The microCT images show that this muscle layer clearly divides into two sections, anterior and posterior, in the squirrel and rat (Fig. 6a,b). Figure 6a also clearly shows the separation of the superficial and deep masseters in axial view, based on variation in the fascicle direction. The anterior part of the deep masseter originates from the rostrum and inserts on the anterior portion of the masseteric ridge, and the posterior part originates further back on the zygomatic arch and inserts on the mandibular angle. Such a division is not so easily discerned from the microCT images of the guinea pig (Fig. 6c) and so, in this specimen, the deep masseter has been reconstructed as a single muscle. In the rat and squirrel, the deep masseter has spread anteriorly on to the rostrum to originate from the masseteric fossa and the widened inferior root of the zygomatic process of the maxilla, as can be seen from the 3D reconstructions (Fig. 7b,c). In the rat, the origin of the deep masseter extends as far as the anterior margin of the maxilla, and in the squirrel, beyond this point on to the premaxilla. In the guinea pig (Fig. 7a), this muscle is restricted to the zygomatic arch by the large infraorbital foramen. Thus, the deep masseter is a relatively much smaller muscle in the guinea pig than in the rat and squirrel, where it forms well over
30% of the masticatory muscle volume (see Table 1). This reduced size and restricted origin may explain its lack of separation into anterior and posterior parts.

The division of the deep masseter of the squirrel and rat into anterior and posterior parts is also made by certain other researchers, generally working on sciuromorphs and myomorphs (Yoshikawa and Suzuki, 1969; Weijs, 1973; Ball and Roth, 1995; Thorington and Darrow, 1996; Druzinsky, 2010a). It is less common in studies of the hystricomorphs where there is no expansion of
the muscle on to the rostrum. Woods (1972), working on a number of hystricomorph genera, splits the masseter lateralis profundus into anterior and posterior parts but notes that the separation is difficult to see in *Cavia*. This was certainly the case in this study, the microCT scans giving no indication of such a division in the deep masseter of the guinea pig.

### Zygomaticomandibularis

Hiemae and Houston (1971) do not recognize the zygomaticomandibularis as a separate muscle in the rat, finding it insufficiently distinguishable from the deep masseter. This arrangement is followed in more recent works, such as Byrd (1981), Satoh (1997, 1998, 1999), and Bresin et al. (2000). However, the microCT images of the rat in this study show a distinct division between the deep masseter and zygomaticomandibularis (Figs. 5 and 6b), and so the latter has been retained as a discrete muscle (in line with Turnbull, 1970; Weijs, 1973). It is also clearly visible as a separate entity in the squirrel (Fig. 8) and guinea pig (Fig. 3).

The innermost layer of the masseter runs between the zygomatic arch and the dorsal part of the mandible. More specifically, it originates from the medial surface of the zygomatic arch (not only largely on the jugal but also on the parts of the maxilla and squamosal) and inserts on the lateral surface of the lower jaw. In squirrels (Fig. 8), the insertion is in the masseteric fossa, just posterior to the toothrow and ventral and anterior to the mandibular condyle. In rats (Figs. 2 and 9) and guinea pigs (Fig. 10), the zygomaticomandibularis inserts on the lateral crest below the second and third molars and on to the coronoid process. In particular, the microCT images of the guinea pig (Fig. 10) show very clearly the ventromedial orientation of the muscle fascicles between the zygomatic arch and lower jaw. As in the previous layer, the microCT images provide good evidence to justify splitting this muscle into anterior and posterior parts. The anterior part runs ventrally from the medial surface of the jugal to the lateral crest, whereas the posterior part originates on the ventral and medial surface of the zygomatic process of the squamosal and runs anterocaudally to the coronoid process of the mandible. As well as showing different orientations of their muscle fascicles, the two parts are also clearly separated by the masseteric nerve (Figs. 8 and 9).

In the rat and the guinea pig, there is an anterodorsal expansion of the zygomaticomandibularis into the orbit and through the infraorbital foramen to take an origin from the rostrum. The expansion of this muscle is relatively large in the guinea pig (Fig. 11a), extending through the grossly enlarged infraorbital foramen to take a large origin on the premaxilla as well as on the maxilla. However, the rostral origin is much smaller in the rat (Fig. 11b), restricted to the area of the maxilla dorsal to the mesoskeletal fossa (the origin of the anterior deep masseter). This expansion of the zygomaticomandibularis is completely absent in the squirrel (Fig. 11c). In both the rat and guinea pig, the infraorbital part of the zygomaticomandibularis inserts into a fossa at the anterior end of the lateral crest, ventrolateral to the first cheek tooth. Figure 6b shows all three parts of the zygomaticomandibularis of the rat, clearly separated from one another, in axial view. Overall, the zygomaticomandibularis makes up a small part of the masticatory musculature—less than 10% in the rat and squirrel (see Table 1). It is slightly greater in the guinea pig (15%) owing to the large infraorbital portion in this species.

In a few studies (generally on hystricomorph rodents), an extra section of this muscle has been distinguished running almost horizontally from the lateral jugal fossa of the zygomatic arch to the postcondyloid process on the mandible. Woods (1972) and Wood (1974) refer to it as the “masseter lateralis profundus, pars posterior, deep division”; subsequent studies (Woods and Howlands, 1979; Woods and Hermanson, 1985; Offermans and De Vree, 1989; Olivares et al., 2004) use the less cumbersome “posterior masseter.” Druzinsky (2010a) describes the posterior masseter in *Aplodontia rufa* and *Marmota monax*, but notes that, owing to its more vertical course in these rodents, it is very difficult to separate it from the posterior zygomaticomandibularis. No evidence of a separate posterior masseter was found in any of the contrast-enhanced microCT images used in this study.

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**Fig. 7.** Left lateral view of 3D reconstructions of the skull, mandible and masticatory muscles of (A) guinea pig, (B) rat and (C) squirrel with the superficial masseter and lateral temporalis removed. adm, anterior deep masseter; dm, deep masseter; iozm, infraorbital part of zygomaticomandibularis; mt, medial temporalis; pdm, posterior deep masseter; t, temporalis. All scale bars: 5 mm.
Fig. 8. Coronal μCT slice through head of squirrel. azm, anterior zygomaticomandibularis; dm, deep masseter; ep, external pterygoid; ip, internal pterygoid; lt, lateral temporalis; m, mandible; mn, mandibular nerve; mt, medial temporalis; pzm, posterior zygomaticomandibularis; sm, superficial masseter; za, zygomatic arch. Scale bar: 5 mm. Slice position shown by dashed line.

Fig. 9. Coronal μCT slice through head of rat. azm, anterior zygomaticomandibularis; dm, deep masseter; ep, external pterygoid; ip, internal pterygoid; lt, lateral temporalis; m, mandible; mn, mandibular nerve; mt, medial temporalis; pzm, posterior zygomaticomandibularis; sm, superficial masseter; za, zygomatic arch. Scale bar: 5 mm. Slice position shown by dashed line.
study, in agreement with Weijs (1973), Cooper and Schiller (1975), and Ball and Roth (1995).

Temporalis

The temporalis muscle is a relatively small muscle, compared with the masseter, in the squirrel and guinea pig, forming around 10% of muscle mass (see Table 1). However, it is a much larger component in the rat, accounting for over a quarter of jaw-closing musculature (as also noted by Schumacher and Rehmer, 1962). This comparison is most clearly illustrated in axial view (see Fig. 12), in which the temporalis muscles of the rat are shown to be at least double in mediolateral width relative to skull size. In rats and squirrels, the microCT images show a division of the temporalis into medial and lateral parts (Figs. 2 and 8). The medial temporalis takes its origin from the lateral surface of the cranium, extending rostrocaudally from the frontal-parietal suture to the lambdoid crest, and dorsoventrally from the temporal ridge to the external auditory meatus and zygomatic process of the squamosal. The muscle fascicles from this wide origin converge to a small region on the medial surface of the mandible between the retromolar fossa and the coronoid process. The lateral temporalis takes its origin from the anterior half of the fascia covering the temporalis and inserts on the coronoid process. It is a relatively large component in the rat, extending widely over the surface of the temporalis (Fig. 4b) but much more limited in origin in the squirrel (Fig. 4c). This division between medial and lateral was not visible in the guinea pig microCT images and so the temporalis has been reconstructed as a single muscle in this specimen (Fig. 4a). However, the temporal region of the guinea pig microCT scan is poorly resolved compared with that of the squirrel and rat (Fig. 12)—a separation of the guinea pig temporalis may yet exist and be visible with higher quality image data.

The division of the temporalis into two parts has been made by many authors (Woods, 1972; Weijs, 1973; Woods and Howlands, 1979; Ball and Roth, 1995; Thorington and Darrow, 1996; Druzinsky, 2010a). Some previous research (Woods, 1972; Weijs, 1973) has separated the ventral most fibers of the temporalis to create a third division, the posterior temporalis (called the suprazygomatic by Druzinsky, 2010a). This usually consists of those fibers taking origin from the caudal region of the squamosal (Weijs, 1973), or in some cases, just those fibers taking origin from the zygomatic process of the squamosal (Woods, 1972; Hautier and Saksiri, 2009; Druzinsky, 2010a). Hautier (2010) describes a third division of the temporalis in Ctenodactylus but suggests that this is not universal among rodents and may result from lateral displacement of the temporalis owing to the distal position of the eye. Such an eye position and the concomitant third division of the temporalis are also found in the dipodoids (Klingener, 1964). This temporal division was not clearly separable in any of the three specimens under study here.

Internal Pterygoid

This muscle is well-developed in rodents and has a similar morphology in all three species in this study. It is particularly notable in the guinea pig in which it accounts for almost a fifth of the jaw-closing musculature (see Table 1 and Fig. 3). The internal pterygoid originates in the pterygoid fossa posterior to the molar toothrow. In squirrels, it also has an origin on the lateral surface of the pterygoid process. From here, the internal
pterygoid runs ventrolaterally and fans out to make a wide insertion on the medial surface of the angular process, dorsal to the reflected insertion of the superficial masseter (Figs. 3, 8, and 9).

**External Pterygoid**

This muscle, like the internal pterygoid, varies little in its morphology between the three rodents. However, it is a much smaller muscle compared with its internal counterpart, forming just 3%–4% of the jaw-closing musculature (see Table 1). It originates along the ventral margin of the skull in the orbitotemporal region on the alisphenoid bone and lateral pterygoid process. It runs posterodorsally to insert on the medial condyloid process, just below the condyle (Figs. 3, 8, and 9).

**DISCUSSION**

The masticatory musculature of rodents is particularly unusual compared with that of other mammalian groups, especially with regard to the morphology of the masseter complex. It has been the subject of numerous studies over the years, and yet there still exist many disagreements and inconsistencies between these works, not only in nomenclature but also in anatomical detail as well. In particular, the description of the musculature of the rat by Hiiemae and Houston (1971), in which only two layers of the masseter are recognized (the zygomaticomandibularis being indistinguishable from the deep masseter in their view), is at odds with most other morphological work on rats (e.g., Turnbull, 1970; Weijs, 1973), and indeed other rodents (e.g., Wood and White, 1950; Wood, 1965; Woods, 1972; Gorniak, 1977; Ball and Roth, 1995; Thorington and Darrow, 1996; Druzinsky, 2010a). Yet, Hiieima and Houston's (1971) work has still been used as the basis for muscle anatomy in several other more recent articles (e.g., Byrd, 1981; Satoh, 1997, 1998, 1999; Bresin et al., 2000). The current study has highlighted these differences by presenting a review of previous work alongside morphological descriptions of the masticatory muscles of the squirrel, rat, and guinea pig, based on the new imaging technique of contrast-enhanced microCT.

Three distinct layers of the masseter (superficial masseter, deep masseter, and zygomaticomandibularis) have been demonstrated in all three rodents under study (contra Hiiemae and Houston, 1971). The deep masseter has been shown to divide clearly into anterior and posterior parts in the squirrel and rat, but it is a much smaller muscle in the guinea pig and has not been separated into two portions. Druzinsky (2010b) found that the enlargement of the anterior deep masseter (i.e., sciurromorphy) increases the efficiency of biting at the incisors, so the reduced deep masseter of the guinea pig may indicate that it processes most of its food by chewing at the cheek teeth and spends less time gnawing at the incisors than the squirrel or rat. The diets of the rodents seems to support this inference, with rats and particularly squirrels consuming a great deal more hard food (nuts and seeds) than guinea pigs (Nowak, 1999). However, it should be noted that *Cavia porcellus* has a relatively larger superficial masseter and zygomaticomandibularis. Hence, the masseter forms a similar overall contribution (~70%) to the masticatory musculature in the guinea pig and squirrel (see Table 1). No evidence for the posterior masseter (Woods and Howlands, 1979; Druzinsky, 2010a) was found in any of the three rodents. This is perhaps not surprising as it is not described in any dissections of *Sciurus* or *Rattus* (Turnbull, 1970; Weijs, 1973; Ball and Roth, 1995), and Woods (1972) states that *Cavia* shows the least degree development of this muscle division among the hystricomorphs he studied.

The temporalis muscle was shown to be considerably larger in the rat than in the squirrel and the guinea pig.
Hiiemae (1971) suggests that the anterior temporalis functions most effectively at the power stroke of mastication, so the enlarged temporalis of the rat may indicate a powerful molar bite in this species. The temporalis was split into two sections (lateral and medial) in the rat and squirrel, but such a division was not clear in the guinea pig. It has been suggested that the two parts of the temporalis operate independently, the lateral (or anterior) part serving to stabilize and elevate the mandible (Hiiemae, 1967). So, in rodents in which the mode of mastication is mostly propalinal grinding, as opposed to crushing, the lateral temporalis is reduced (Klingener, 1964; Woods, 1972). Hence, the absence (or extreme reduction) of the lateral temporalis in the guinea pig could be an adaptation to a lifestyle in which the action of the molars is almost always grinding and hardly ever crushing. In contrast, in squirrels and rats, where propalinal chewing is much less important, the lateral temporalis is present, although its origin on the fascia overlying the medial temporalis is slightly more restricted in *Sciurus* in this study than is indicated in any of the squirrel genera studied by Ball and Roth (1995) or Thorington and Darrow (1996).

The internal pterygoid was shown to be particularly large in the guinea pig, compared with the rat and squirrel. Contraction of this muscle produces lateral movement (Hiiemae, 1971), which suggests that the guinea pig molar bite has a greater lateral component than that of the other two rodent species. Previous electromyographical studies indicate that this is indeed the case: although there is a lateral component to the rat molar bite, it appears to be small (Hiiemae and Ardran, 1968; Hiiemae, 1971), whereas Byrd (1981) noted a strong lateral component in the guinea pig masticatory cycle. However, the lateral component of guinea pig mastication is still relatively small when compared with other hystricognaths, particularly those in the Octodontioidea (Vassallo and Verzi, 2001). This is largely due to the mandibular morphology in the Caviidae, specifically a low mandibular condyle and a distally positioned angular process.

We confirm that staining of biological specimens with iodine solution has enabled visualization of both the hard and soft tissues of the skull using microCT imaging (Metscher, 2009; Jeffery et al., 2011). In this study, the contrast-enhanced microCT technique has been particularly useful for examining the zygomaticomandibularis muscle. Because of its origin on the medial surface of the zygomatic arch, it is very difficult to dissect out—the superficial and deep masseters have to be removed and part of the zygomatic arch has to be cut away. However, the zygomaticomandibularis and its spatial relationship with the other masticatory muscles, the skull, and lower jaw can be seen very clearly with the aid of contrast-enhanced microCT (see Figs. 1–3, 8, 9). The enhanced microCT also enables the creation of three-dimensional reconstructions (Figs. 4, 7, and 11) and measurements of, for example, muscle mass (see Table 1). It is felt that contrast-enhanced microCT will become an important tool in the study of soft tissue morphology and will be of particular interest to researchers in the field of biomechanical analysis, particularly finite element analysis and multibody dynamic analysis, for whom accurate knowledge of muscle geometry is essential.

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**Fig. 12.** Transverse μCT slices through head of (A) squirrel, (B) rat and (C) guinea pig. adm, anterior deep masseter; iozm, infraorbital part of zygomaticomandibularis; lt, lateral temporalis; mt, medial temporalis; t, temporalis. All scale bars: 5 mm. Slice positions shown by dashed lines.
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