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Sleep quantitation in common marmoset, cotton top tamarin and squirrel monkey by non-invasive actigraphy

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Abstract

Sleep quantitation data on the Neotropical primate species, apart from the squirrel monkey, are still sparse. As such, we have quantitated sleep in the common marmosets (*Callithrix jacchus*), cotton top tamarins (*Saguinus oedipus*) and squirrel monkeys (*Saimiri sciureus*) reared in one primate facility simultaneously, by non-invasive actigraphy. The range in total sleep time/24 h measured for male adult common marmosets, cotton top tamarins and squirrel monkeys were 713–793 min ($n=4$), 707–889 min ($n=4$) and 459–475 min ($n=2$) respectively. The range in sleep episode length /12 h dark phase for marmosets, tamarins and squirrel monkeys were 21–52 min ($n=3$), 10–28 min ($n=4$) and 9–15 min ($n=2$) respectively. Since vigilance is a critical evolutionary adaptive feature of predator avoidance among Callitrichid monkeys and squirrel monkeys, the shorter ranges in sleep episode length recorded, even under captivity, in this study could be interpreted as probable indicators of such vigilance behavior during the rest phase. We hypothesize that the vigilance behavior when it exists during a primate's active phase should also prevail when it is at rest (sleep). This hypothesis deserves additional testing in female Callitrichid monkeys.
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Keywords: Actigraphy; Callitrichidae; *Callithrix jacchus*; Cebidae; *Saguinus oedipus*; *Saimiri sciureus*; Sleep episode length; Vigilance

1. Introduction

It is hardly debatable that simultaneously conducted comparative biochemical studies in multiple non-human primate species provide dividends in characterizing the phylogenetic links among evolutionary kins (Welsh and Walker, 1972, 1973; McGeachin and Akin, 1982; Roger et al., 1993; Croll et al., 1993; Fukuhara and Kageyama, 2005). However, due to restraints in space, time, funds and biased concerns of activists espousing animal rights, conducting comparative, prospective physiological studies simultaneously in more than one species of primates is becoming less feasible to plan and execute. Despite such a bottleneck, curiosity and need to quantitate the yet unknown details on the sleep behavior of three prominently used laboratory primates belonging to Cebidae family prompted us to initiate this study at our primate facility in Inuyama, Japan.

Sleep behavior in mammals, according to Zepelin (1989), can be defined by two criteria: (1) sustained quiescence in a species-specific posture accompanied by reduced responsiveness to external stimuli, and (2) quick reversibility to the wakeful condition, which distinguishes sleep from coma and hypothermic states such as hibernation and torpor. In addition, a third criterion representing characteristic changes in the electroencephalogram (EEG) is also considered as a useful validatory component of sleep. Of all the behaviors exhibited by the non-human primates (hereafter, referred to as primates), sleep behavior is rather difficult to quantitate (Siegel, 2005), especially the validation by EEG under acceptable standards of scientific rigor.

As of now, sleep behavior has been quantitated in only around 5% of the 234 extant primate species (Campbell and Tobler, 1984; Zepelin, 1989). Paucity of sleep quantitation data in the primate field studies is understandable. Absence of natural light during the sleep phase of primates is a prime hindrance for observation. To further compound the issue, artificial light disturbs the sleep behavior of the focal animals. The fatigue factor of the human observer in nights which leads

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to sub-par and erroneous data collection cannot be overlooked. One should also not underestimate the inherent danger from other poisonous and predatory animals in the milieu which may affect the concentration of the researcher. Thus, sleep behavior studies have to depend more on primates reared in captivity.

Common marmosets (*Callithrix jacchus*) and cotton top tamarins (*Saguinus oedipus*), among the New World monkeys belonging to Callitrichidae, have gained prominence as cost-effective and lab-worthy primates for experimental use in captivity (Stellar, 1960; Poole et al., 1999). While common marmosets are endemic to Brazil, the cotton top tamarins are endemic to Colombia. Though still widespread in the Pernambuco region of Brazil, the wild *C. jacchus* populations have been suffering from severe depletion and decline due to habitat destruction (Rylands, 1993; Norconk et al., 1996). As a cumulated consequence of habitat loss, hunting and illegal capture for pet trade, a harsher vulnerable-threatened fate is now faced by wild *S. oedipus* populations in its native north western Colombian habitat, bordering the Caribbean Sea — between the eastern bank of Atrato river and western banks of Cauca and lower Magdalena rivers (Mast et al., 1993; Defler, 2004). The extent of the spread of *S. oedipus* to the south is believed to be 1500 m altitudinal limit into the Andean foothills (Mast et al., 1993).

Past field studies on *C. jacchus* and *S. oedipus* monkeys and their rather unique ecological role among the anthropoid primates have been reviewed (Sussman and Kinzey, 1984; Rylands, 1993; Defler, 2004). However, there is paucity of information on the sleep behavior of *Callithrix* and *Saguinus* genera living in the wild, and what is available were based on quasi-quantitative, opportunistic and/or naturalistic observations (Hershkovitz, 1977; Dawson, 1979; Heymann, 1995; Day and Elwood, 1999). The squirrel monkey (*Saimiri sciureus*), a New World monkey belonging to Cebidae, also continues to be one of the popular primate species for studies in neuroscience and pharmacology, since its introduction into aerospace medical research and animal space flight in 1958 (Beischer, 1968). As such polysomnography-derived data on sleep–wake cycles and biorhythm of squirrel monkeys have been published from a few laboratories (Adams and Barratt, 1974; Wexler and Moore-Ede, 1984, 1985, 1986; Erny et al., 1985; Breton et al., 1986; Edgar et al., 1993; Klerman et al., 1999, 2000). A couple of the early studies on sleep quantitation using polysomnography in squirrel monkeys (Adams and Barratt, 1974; Breton et al., 1986) were limited to 10–12 h.

Though polysomnography still remains as the 'gold standard' for sleep quantitation measurements, it is a highly invasive technique. Using brain and muscle electrodes in primates, seated in a restraint chair, has come under serious criticism as well since 1980s. The inability to sustain the recording for days is another demerit for polysomnography. Thus, quantitative sleep data recorded by non-invasive methods such as actigraphy have gained acceptance lately in understanding the sleep architecture of different primate species, including humans (Zhdanova et al., 2002; Ancoli-Israel et al., 2003; Sri Kantha and Suzuki, in press). As such, our objectives were two fold: (1) to quantitate sleep in the common marmosets and cotton top tamarins reared in our primate facility simultaneously, by non-invasive actigraphy, to fill the currently existing void on sleep literature of *C. jacchus* and *S. oedipus* populations, and (2) to calibrate the actigraphy sleep measurement in *S. sciureus* monkey with the previously reported polysomnographic sleep measurement for this species.

2. Materials and methods

2.1. Animals and housing

The age composition and masses of study subjects reared at the Primate Research Institute facility and used in the experiments are provided in Table 1. Male members of common marmosets (*C. jacchus*, $n=5$), cotton top tamarins (*S. oedipus*, $n=4$) and squirrel monkeys (*S. sciureus*, $n=2$) were used in this study. As indicated in Table 1, excluding one tamarin (So 105), all five marmosets and three tamarins were pair-housed with either a female mating partner or sibling/half-sibling or parent/offspring, in metal cages of dimension, 70 cm × 70 cm × 150 cm. The two squirrel monkeys shared a stainless group cage (1.2 m × 1.2 m × 2.1 m) with two adult females and a young male. This group cage had access to a sun-room with adequate vertical space (24 m × 28.5 m × 77.5 m) incorporated with diverse small vegetation including vines, small water facility and enrichment accessories like ropes and wood planks as multiple level perches. Mention should be made that when squirrel monkeys were studied in May 2005, the natural daylight extended to 14 h, and as such, the

Table 1
Basic details of the male study subjects and the duration of sleep quantitation

Monkey ID	Age at experiment[a] (years)	Weight (g)	Duration of sleep quantitation (days)
Common marmoset[b]			
Cj 62	10	412	7
Cj 98	8	440	7
Cj 123	6	363	7
Cj 133	6	277	7
Cj 174	0.8	266	7
Cotton top tamarin[c]			
So 105	14	425	8
So 133	11	500	8
So 165	7	473	8
So 173	6	533	8
Squirrel monkey[d]			
Ss 114	13	927	3
Ss 115	13	954	3

[a] All monkeys were born in captivity.
[b] Cj 62 and Cj 98 are half-siblings sharing the same cage. Cj 123 was pair-housed with a mating partner. Cj 133 and Cj 174 are parent and male offspring, sharing the same cage.
[c] So 105, being senile, was housed in a single cage. So 133 was pair-housed with a mating partner and their baby offspring. So 165 and So 173 are siblings sharing the same cage.
[d] Ss 114 and Ss115 shared a group cage with two females and another male, age ranging from 5 to 10 years.

complete dark phase during the study period was shortened to 10 h.

While cotton top tamarins and squirrel monkeys occupied the same room, the cages of common marmosets were in a separate room. Thus, the light intensity of the two rooms varied. Alternating 12 h light (6:00 h–18:00 h) and 12 h dark (18:00 h–6:00 h) phases prevailed in the housing facility. The light intensities at the bottom of the cages, 1 m above the ground during the light phase were as follows; 60–150 lux (marmosets), 800–1250 lux (tamarins) and 440–460 lux (squirrel monkeys), as routinely checked by an illuminance meter (TopCon IM-5, Tokyo).

The pair-housing cages of marmosets and tamarins were designed in such a way that though visual contacts among the conspecifics occupying separate cages were impermissible, unrestricted vocal contacts could be heard by the conspecifics around the clock. All monkey cages were provided with commercial food pellets for New World Monkeys (25.1 g protein and 10.6 g lipid/100 g diet) and water ad libitum. In addition, monkeys also received sliced fruit portions and insects as additional food supplements on a daily basis. The room temperature and relative humidity were maintained by automatic controls at 25±1 °C and 50–60% respectively.

2.2. Experiments and measurements

Activity–sleep patterns of the monkeys were monitored via actigraphy [Actiwatch AW 64 model-MINIMITTER, Mini Mitter Company, Bend, Oregon, USA; containing 64KB of on-board memory] as described previously (Sri Kantha and Suzuki, in press). Briefly, the actiwatch (weighing 17 g), preset to collect activity–rest data of individual monkey with a sampling rate of 32 Hz and a sampling epoch of 1 min, was suspended in a ribbon, and positioned on monkey's back following anesthesia with ketamine HCl (10 mg/kg body weight; Sankyo, Tokyo). For each experimental subject, longitudinal data on activity–rest behavior, daily total sleep time (TST) and sleep episode length (SEL) were collected before removal of actiwatch from monkey's neck. Data collection periods for marmosets, tamarins and squirrel monkeys were 7 days, 8 days and 3 days respectively. Accumulated data were transferred to the computer, via Mini Mitter Actiwatch Reader through an RS-232 Serial Port and Activity Sleep® Activity Monitoring Software, version 3.3 [Mini Mitter Company].

As per the operational definition of sleep in actigraphy, in the absence of any force made by the subject, which exceed 10 mg

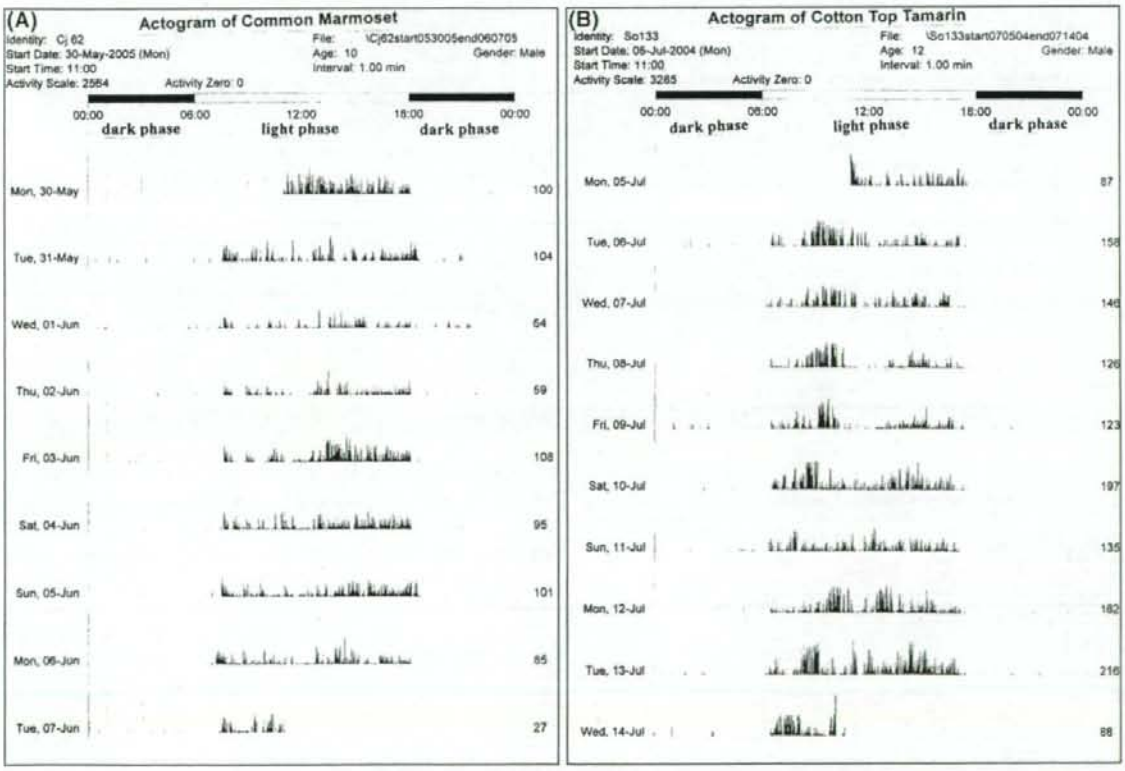

Fig. 1. Representative actograms of two Callitrichid monkeys studied under captive condition. (A) A male common marmoset (*Callithrix jachchus*), aged 10 years; (B) a male cotton top tamarin (*Saguinus oedipus*), aged 12 years. The number indicated in each row of the right end of both panels refers to mean Actiwatch activity counts per a 24-h period. The almost-blank spaces recorded during the dark phase (18:00 h–06:00 h) equates to the recurrent rest (sleep) cycle.

in any direction during the measuring period (counted as epoch of 1-min duration), the animal was assumed to be at rest — i.e., sustained quiescence in a species specific posture, and thus equated to sleep in somnology studies (Campbell and Tobler, 1984). The activity–rest phases of the animal were quantitated in an arbitrary unit called Actiwatch (AW) activity count, where any omnidirectional motion by the animal with a minimal resultant force of 10 mg was quantitated by the instrument with reference to degree and speed of motion. These parameters are converted to produce an electrical current that varied in magnitude. This derived algorithm is referred to as activity count, which is instrument specific.

During the experimental period, female cage mates of the experimental monkeys of all three species were also tagged with Actiwatches for experimental rigor. All experiments were carried out following approval from the Research Committee of the Primate Research Institute, and according to this Institute's Guidelines for the Care and Use of Laboratory Primates. Data collection began in April 2004 and ended in June 2005.

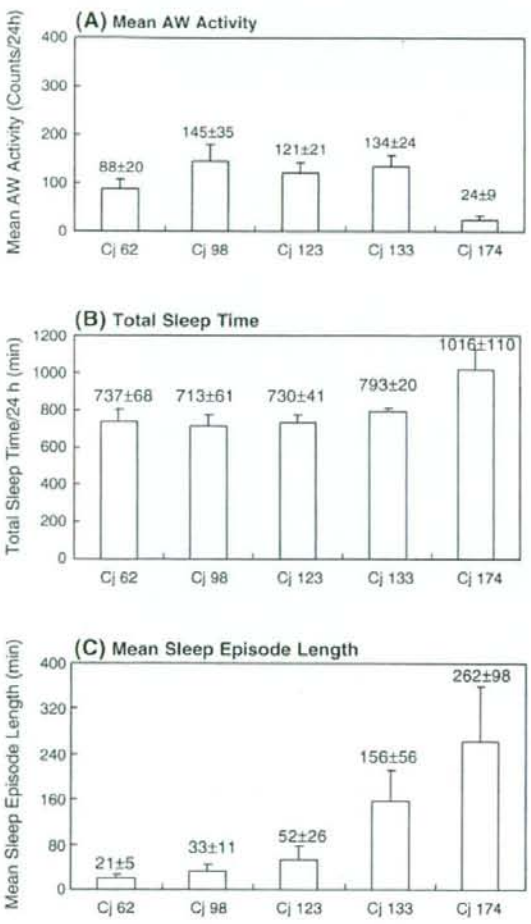

Fig. 2. Sleep Quantitation in male common marmosets. (A) Daily activity, (B) daily total sleep time, (C) sleep episode length during the dark phase. Results are also indicated in numbers (mean±SD) above each histogram.

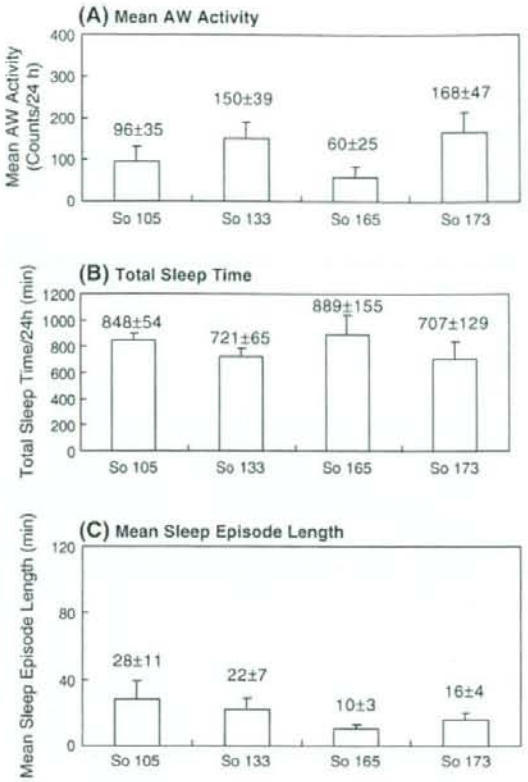

Fig. 3. Sleep Quantitation in male cotton top tamarins. (A) Daily activity, (B) daily total sleep time, (C) sleep episode length during the dark phase. Results are also indicated in numbers (mean±SD) above each histogram.

3. Results

3.1. Activity

Representative actograms of common marmoset (Cj 62, aged 10 years) and cotton top tamarin (So 122, aged 12 years) are presented in panels A and B of Fig. 1 respectively. The number indicated at the right end of each row of both panels refers to Actiwatch (AW) mean activity counts per a 24-h period. The almost-blank spaces recorded during the dark phase (18:00 h–06:00 h) indicate the recurrent rest (sleep) cycle. The daily activity levels, measured as mean AW activity counts, of marmosets, tamarins and squirrel monkeys are shown in the panel A of Figs. 2–4 respectively. The mean values of activity counts for four adult marmosets ranged between 88 and 145 (Fig. 2A). However the activity value (mean±SD) of 24±9 recorded for the 10-month-old juvenile marmoset (Cj 174) was 5.6-fold lower to the activity value (134±24) of its 6-year-old parent sharing the same cage; this

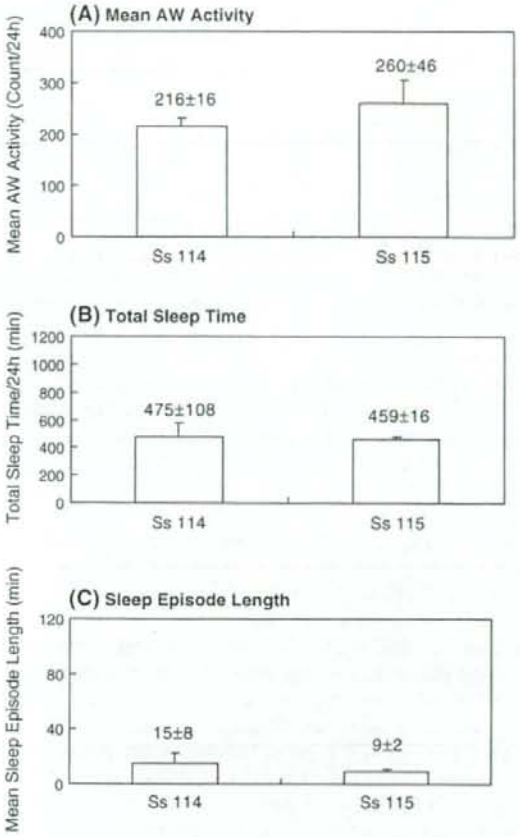

Fig. 4. Sleep Quantitation in male squirrel monkeys. (A) Daily activity, (B) daily total sleep time, (C) sleep episode length during the dark phase. Results are also indicated in numbers (mean±SD) above each histogram.

result, indicating the developmental immaturity of the juvenile marmoset was not unusual. The mean values of activity counts for four adult tamarins, with a range from 60 to 168 (Fig. 3A) more or less overlapped with the range determined for the adult marmosets. Compared to the activity counts recorded for both marmosets and tamarins, the mean daily activity count of 238 for two squirrel monkeys was markedly higher. This result is also reasonably convincing in terms of the difference in physical stature, body weight and athletic capabilities between the two Callitrichid monkeys and the squirrel monkey.

3.2. TST

The quantitated daily TST of marmosets, tamarins and squirrel monkeys are presented in the panel B of Figs. 2–4 respectively. While the daily TST of four adult marmosets ranged between 713 and 793 min (Fig. 2B), the daily TST of four adult tamarins ranged between 707 and 889 min (Fig. 3B). Though the TST range of tamarins was broader by 102 min, and the mean (±SD) TST values for marmosets and tamarins were 743±35 min and 791±91 min respectively, the overlap in daily sleep time between these two species is markedly obvious. Fig. 2B also indicates that the daily TST of juvenile marmoset (Cj 174) exceeded that of its parent (Cj 133) sharing the same cage by 223 min; and this was again an expected phenomenon, considering the physical immaturity and lower activity profile of the juvenile monkey. However, the mean TST values of 467 min recorded for the two squirrel monkeys (Fig. 4B) was 4–5 h lower in comparison to the mean TST values of adult marmosets and tamarins.

3.3. SEL

The quantitated SEL during the dark phase (18:00 h–06:00 h) of marmosets, tamarins and squirrel monkeys are shown in the panel C of Figs. 2–4 respectively. The mean SEL of four adult marmosets showed an unusually long range, from 21 min to 156 min (Fig. 2C), though this range when revised to accommodate the numbers obtained for three monkeys (namely Cj 62, Cj 98 and Cj 123) would become narrower between 21 min and 52 min. This revision is rather reasonable, because Cj 133 monkey – as per the experimental design – shared the cage with its juvenile offspring Cj 174, whose SEL was an unusual 262 min. In contrast to the marmosets, as Figs. 3C and 4C indicate, the SEL ranges of four tamarins (10–28 min) and two squirrel monkeys (9–15 min) were markedly shorter. As a sleep parameter, SEL appears to be species specific and probably less influenced by the immediate external environment, in comparison to TST.

4. Discussion

The representative activity–rest pattern of common marmoset, presented in Fig. 1 (panel A) in this study, corroborates well with the previous data figures of Erkert et al. (1986) and Menezes et al. (1993), on the circadian activity rhythms of common marmoset, obtained under comparable experimental protocols. However, we were unable to locate any previous reports with figures on the activity–rest pattern of captive cotton top tamarins, to corroborate the data presented in Fig. 1 (panel B).

To our knowledge, the present study appears to be the only attempt so far, to quantitate two sleep parameters (TST and SEL) of three New World monkey species captively-reared in the same facility, using the same instrument, for a period of ≧3 days. Among the New World monkeys, while sleep quantitation by polysomnography in squirrel monkey has been reported in the past (Table 2), sleep data of Callitrichid monkey species obtained by any objective method, excluding that of Crofts et al. (2001), have been lacking. Even in the squirrel monkeys, the experimental designs of the past polysomnography studies leave much to be desired. In almost all these studies due to experimental constraints, monkeys were restricted to sound-attenuated, individual cages during sleep quantitation. This type of artificial environment is at variant from the natural milieu in which squirrel monkeys rest, occasionally in huddle positions with their conspecifics

(Kaplan, 1977; Mendoza, 1999). As such, sleep quantitation of captive squirrel monkeys living in a simulated natural milieu became a relevant objective for us.

In Table 2 we provide a summary of past sleep quantitation data for common marmosets and squirrel monkeys and have added the results obtained in the present study for these two species as well as cotton top tamarins. In the present study, we have quantitated the daily mean TST and SEL in these three species. Though the natural, wild milieu prevailing in the Neotropical rain forests could not be duplicated, we have attempted to quantitate TST and SEL of the common marmosets, cotton top tamarins and squirrel monkeys under conditions that are mildly reflective of the wild state. Either the breeding pairs/parents and offspring or the siblings were grouped together in cages for marmosets and tamarins. The sleep in two squirrel monkeys was measured in an unrestrained set up, where they were living with three conspecifics in a group cage which had access to a sun-room with adequate vertical space.

It is evident from Table 2 that the TST values of common marmosets (743±35 min) obtained in the present study using actigraphy aligns favorably with the early reports Fitzgerald (1935) and Stellar (1960), based on subjective counting. But the TST values measured by radio telemetry (Crofts et al., 2001) were nearly 3 h lower than that of the present study. Nevertheless, the SEL range of 40–50 min for marmosets reported by Crofts et al. (2001) is in agreement with the SEL range of 21–52 min obtained in the present study for three adult marmosets, namely Cj 62, Cj 98 and Cj 123. Since the present study appears to be the first report on the sleep quantitation in cotton top tamarins, the mean TST (791±91 min) and the SEL range (10–28 min) for this species awaits further confirmation. However, indirect confirmatory support for the validity of TST values obtained in the present study is available. The daily TST values obtained by us for *C. jacchus* (12 h 23 min, mean of 4 adults) and *S. oedipus* (13 h 11 min, mean of 4 adults) are in harmony with the previous determinations of active phase duration in these two species, living in the wild. Castro et al. (2003) have reported that free-ranging *C. jacchus* members living at the Experimental Forestry Station in Northeastern Brazil showed an average duration of the active phase of 11:37 h±13.8 min. Dawson (1979) had determined that for *S. oedipus* members inhabiting the Pacific slope of Panama Canal Zone, the total daily activity time averaged 11 h.16 min±62 min. The durations of daily activity time reported by Castro et al. (2003) and Dawson (1979) for *C. jacchus* and *S. oedipus* respectively, when subtracted from 24 h provide the duration of rest phase for these two species and the derived numbers tally perfectly with the TST values obtained in the present study for *C. jacchus* and *S. oedipus*.

Table 2
Summary of sleep quantitation in three species of New World monkeys

Method	Number and sex [a]	Body weight (g)	Mean TST (min)	SEL range (min)	Authors [b]
Common marmoset					
[Unspecified] [c]	11?	n.r.	780–840	n.r.	1
[Unspecified] [c]	25?	n.r.	~840	n.r.	2
Radio telemetry	4♂+♀	n.r.	569±8	40–50	3
Actigraphy	4♂	277–440	743±35	21–156	This study
	1♂ [d]	266	1016	262	This study
Cotton top tamarin					
Actigraphy	4♂	425–500	791±91	10–28	This study
Squirrel monkey					
Polysomnography	3♂	560–700	593±62	n.r.	4
Polysomnography	4♂	~900	672±12 n.r.		5
Polysomnography	11♂+♀	600–800	329±32 n.r.		6
Polysomnography	5♂	1100–1150	534±40	n.r.	7
Actigraphy	2♂	927–954	467	9–15	This study

TST—total sleep time (mean±SD); SEL—sleep episode length (mean±SD); n.r.—not recorded.

[a] All monkeys of three species, excluding one *C. jacchus* juvenile used in this study, were adults.

[b] Authors: 1—Fitzgerald, 1935; 2—Stellar, 1960; 3—Crofts et al., 2001; 4—Adams and Barratt, 1974 (12 h recording only); 5—Wexler and Moore-Ede, 1985; 6—Breton et al., 1986 (10 h recording only); 7—Edgar et al., 1993.

[c] Most probably subjective counting; the exact number and sex of marmosets observed for sleep quantitation not clearly specified, since these were early attempts on breeding this species under captivity.

[d] The juvenile (Cj 174), aged 10 months, used in this study for the purpose of checking age-related difference in sleep in comparison to its male parent (Cj 133), sharing the same cage.

As for the squirrel monkey, the average TST value of 467 min obtained in the present study by actigraphy lies in between the lower (329±32 min) and upper extremes (672±12 min) of TST values reported by four previous groups using polysomnography (Table 2). But the SEL range for squirrel monkeys have not been recorded in these reports (Adams and Barratt, 1974; Wexler and Moore-Ede, 1985; Breton et al., 1986; Edgar et al., 1993). As such, the validity of the shorter SEL range of 9–15 min for squirrel monkey reported in this study deserves confirmation, under identical experimental set up. Under the experimental set up used in this study where squirrel monkeys were provided with a sun-room and when measurements were made the natural daylight was extended to 14 h, the resulting effect of such shortened dark phase on the diminished TST (in comparison to previous reports by Adams and Barratt, 1974; Wexler and Moore-Ede, 1985; Edgar et al., 1993) and SEL deserve additional attention.

A positive correlation of SEL to the brain weight and encephalization of mammalian species has been suggested (Zepelin, 1989); i.e., mammals with smaller brains have a shorter SEL (for example, house mouse: 11 min, laboratory rat: 11 min, dog: 20 min, and cat: 25 min) than the mammals with larger brains (for example, baboon: 40 min, chimpanzee: 85 min, and human: 90 min). However, since only meager information is currently available on the SEL of different primate species the validity of an exclusive correlation between SEL and brain weight needs further scrutiny. Apart from the brain weight and encephalization process, we believe that SEL is further influenced by parameters such as age, group composition in the surrounding milieu, ovarian cycle in

Table 3
Comparison of sleep measurement methods for non-human primates

Parameter	Polysomnography	Videography	Actigraphy
(1) Validity:	High; due to differential merit in distinguishing non-REM and REM sleep phases	Medium; sleep phases cannot be differentiated	Medium; sleep phases cannot be differentiated
(2) Convenience:	Low; need for surgery, delay from post-surgical recovery, potential for interference of wires and electrodes by dexterous primate	Low; need for post hoc reviewing and data tabulation from tape, camera positioning need alteration as per primate movement	High
(3) Extended recording:	Feasible only for one to few days	Feasible for weeks	Feasible for weeks
(4) Chair/Gadget restraint:	Needed, and rather sub-natural	Not needed	Not needed
(5) Inter-experimenter reliability:	Medium; because of the need for surgery and its restraints	High; no surgery needed	High; no surgery needed

non-REM—non-rapid eye movement sleep; REM—rapid eye movement sleep.

females, parenting status of the primate and last but not the least, inherent evolutionary adaptiveness of the primate against predation pressure. In the present study, the SEL data obtained for parent–offspring marmoset pair (Cj 133 and Cj 174) living in the same cage demonstrates the influence of age and parenting status on SEL. Though Cj 133 is an adult male, its markedly higher SEL (156±56 min) in comparison to the SEL range (21–52 min) of its conspecifics cohabiting with adults in their respective cages deserves notice.

Vigilance, a critical evolutionary adaptive feature of predator avoidance, is exhibited by Callitrichid monkeys (Caine, 1986, 1987; Ferrari and Ferrari, 1990; Price et al., 1991) and squirrel monkeys (Biben et al., 1989) during their active phases, even if they live under captive conditions. Vigilance has been generally defined as a behavioral 'state of readiness [in animals] to detect specific unpredictable events in the environment' (Harre and Lamb, 1986). According to Treves (2000), the definitions of vigilance contributed by primate behavioral scientists are rather non-uniform because 'many primate studies use idiosyncratic sampling rules'. We also concur with Treves (2000) that vigilance measurement is further compounded by the limitations in methodology to simultaneously detect exhibited multiple modes of vigilance by primates, representing the visual, auditory and olfactory senses. As such, within the boundaries of our recording technique used in this study, the shorter SEL ranges recorded for the cotton top tamarins (10–28 min) and squirrel monkeys (9–15 min) in this study could be interpreted as linked to inherited vigilance behavior. We hypothesize that the vigilance behavior when it exists during a primate's active phase should also prevail when it is at rest (sleep) and the shorter SEL ranges are probable indicators of such vigilance behavior. This hypothesis deserves additional testing in Callitrichid monkeys of female sex as well.

The use of actigraphy as a viable method of sleep quantitation in non-human primates deserves scrutiny. While polysomnography is still considered as the 'gold standard' for sleep quantitation measurements, its many drawbacks also deserve recognition (Table 3). These drawbacks contribute much to the loss of rigor in the gathered data, especially in case of highly intelligent, dexterous non-human primates. Absence of accurate stereotaxic brain atlases further add to the difficulty in measuring the sleep of marmoset and tamarin monkeys via polysomnography. To the best of our knowledge, a stereotaxic brain atlas for cotton top tamarin is still unavailable. Even the available stereotaxic brain atlas for common marmoset by Stephan et al. (1980) was based on the observations made on only 5 adult male marmosets, and carries a disclaimer stating, "This stereotaxic atlas should not be considered as a comprehensive presentation and classification of the cyto and myeloarchitectonics of the brain of the common marmoset monkey. Although a great effort was exerted to identify completely the cellular aggregates which are visible on the plates several grey masses could not be labeled with certainty." As such, until the availability of reliable stereotaxic brain atlases of common marmoset and cotton top tamarin, accurate sleep measurements using polysomnography appear impractical.

To conclude, in order to determine normative values on sleep parameters, in this study we have quantitated TST and SEL for males of common marmosets, cotton top tamarins and squirrel monkeys. These data add to our recent findings on the sleep parameters of owl monkey, *Aotus* species (Sri Kantha and Suzuki, in press). Taken together, these results may be of value (a) to evaluate the functions of sleep among Neotropical primates in phylogenetic and interspecies context (Siegel, 2005), and (b) to aid the protocols of the conservation programs targeted towards marmosets and tamarins in the wild (Kleiman, 1977; Rylands, 1993, Defler, 2004). Data obtained in a companion study in which we have quantitated the TST and SEL for females of common marmosets and cotton top tamarins will be presented separately.

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Descent of the hyoid in chimpanzees: evolution of face flattening and speech

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Abstract

The human supralaryngeal vocal tract develops to form a unique two-tube configuration with equally long horizontal and vertical cavities. This anatomy contributes greatly to the morphological foundations of human speech. It is believed to depend on the reduced growth of the palate and on the developmental descent of the larynx relative to the palate. Anatomically, the descent of the larynx is accomplished through both the descent of the laryngeal skeleton relative to the hyoid and the descent of the hyoid relative to the palate. We have studied the development of three living chimpanzees using magnetic resonance imaging. Our previous study showed that, as in humans, chimpanzees show rapid laryngeal descent, with changes in the relative proportion of the vocal tract during early infancy. However, this is not accompanied by the descent of the hyoid relative to the palate, although it is achieved with the descent of the laryngeal skeleton relative to the hyoid. Here, we show that subsequently the chimpanzee hyoid also descends to maintain the rapid descent of the larynx, similarly to humans. We argue that the descent of the larynx probably evolved in a common ancestor of extant hominoids, originally to confer an advantage via a function unrelated to speech. Thus, the descent of the larynx per se is not unique to humans, and facial flattening was probably the major factor that paved the way for speech in the human lineage.
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Keywords: Descent of the larynx; Vocal tract; *Pan troglodytes*; MRI; Longitudinal study

Introduction

The evolution of human speech has attracted much interest to better understand the evolution of language. Human speech has the distinct feature that humans can regularly utter several phonemes—including vowels and consonants—sequentially and rapidly in a short, single exhalation. It must be noted that speech per se is not the same as language and does not necessarily reflect the high intelligence of humans. However, this sophisticated feature of speech allows humans to turn much information that is encoded by language in the brain into sounds and to communicate it to others rapidly and efficiently (P. Lieberman, 1984). Therefore, even if language and speech arose independently in the human lineage, an understanding of the evolution of speech would shed light on the evolution of language with which we are endowed today.

Theories of speech physiology and acoustics demonstrate that humans have a unique anatomy of the supralaryngeal vocal tract (SVT) that underlies its sophisticated manipulation in the production of speech. Humans and nonhuman mammals basically make use of the same machinery for speech and vocalization: the lungs generate sound power, the vocal folds in the larynx comprise the sound sources, and the SVT resonates the sources to generate voiced sounds with some bands of the formant frequencies, e.g., vowels in speech (Fant, 1960; P. Lieberman and Blumstein, 1988; Titze, 1994; Stevens, 1998; Fitch and Hauser, 2003; Riede et al., 2005). The

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distribution pattern of the formants defines the different kinds of vowels that form the platform for vocal communication. These are determined by the resonance properties of the SVT and these properties in turn are dictated by the volumetric topology of the tract, which can be estimated from a function of the sequential cross-sectional area along the tract. The SVT in most mammals, including humans, is principally composed of two cavities: the horizontal oral and vertical pharyngeal cavities designated here the SVT_H and SVT_V, respectively. In nonhuman mammals, the SVT_V is much shorter than the SVT_H (Negus, 1949; P. Lieberman, 1984; Laitman and Reidenberg, 1993; Dyce et al., 1996). Their epiglottis, which is attached to the thyroid cartilage of the laryngeal skeleton, maintains contact with the velum and prevents the SVT_V from facing the movable tongue. The tongue is also long in the horizontal direction, fitting this configuration. Although this anatomy allows the oral cavity to function as a single resonator, it prevents the pharyngeal cavity from contributing much in that capacity (P.H. Lieberman et al., 1969; P. Lieberman, 1984; Fitch, 2000a; Fitch and Hauser, 2003). Thus, nonhuman mammals have physical constraints to any rapid sequential modification of the cross-sectional areas of the SVT. In contrast, adult humans have an equally long SVT_H and SVT_V. Their epiglottis is separated from the velum, and this produces a long oropharyngeal region facing the dorsal surface of the tongue, rostral to the laryngopharyngeal region that faces the epiglottis (P. Lieberman, 1984; Crelin, 1987; Zemlin, 1988; Titze, 1994). The vertical dimension of the tongue is almost equal to the horizontal dimension to fit this configuration, and the internal musculature of the tongue makes the surface highly mobile (Takemoto, 2001). In anatomical terms, these features allow the shapes of the SVT_H and SVT_V to be sequentially and rapidly modified by tongue movements, semi-independently of each other (P.H. Lieberman et al., 1969; P. Lieberman, 1984; Fitch, 2000a; Fitch and Hauser, 2003). Thus, humans are capable of extensive modification of the resonant properties of the SVT, which in turn modify the laryngeal sounds, forming the complex sequential phonemes of speech in a single, short exhalation.

In newborn humans, the larynx is positioned close to the palate so there is little vertical pharyngeal space (Negus, 1949; P. Lieberman, 1984; Crelin, 1987; D.E. Lieberman et al., 2001). However, the major descent of the larynx causes the epiglottis to descend relative to the velum (Negus, 1949; Roche and Barkla, 1965; Sasaki et al., 1977; Crelin, 1987; Westhorpe, 1987; Fitch and Giedd, 1999; Vorperian et al., 1999, 2005). This establishes a long oropharyngeal space, rostral to the laryngopharyngeal region in the SVT_V. Thus, the SVT_V lengthens rapidly compared with the SVT_H, and can function as a resonance tube of equivalent volume (P. Lieberman, 1984; Crelin, 1987; Titze, 1994; Fitch, 2000a).

The laryngeal skeleton is suspended from the hyoid apparatus, and the hyoid is in turn suspended from the mandible and cranial base by muscles and ligaments (Zemlin, 1988). Anatomically, two processes accomplish the descent of the larynx: the descent of the laryngeal skeleton relative to the hyoid, and the descent of the hyoid relative to the palate. This process has been evaluated with detailed measurements on humans from X-ray photographs (D.E. Lieberman and McCarthy, 1999; D.E. Lieberman et al., 2001) and magnetic resonance imaging (MRI; Fitch and Giedd, 1999; Vorperian et al., 1999, 2005). We have studied the development of three living chimpanzees using MRI. Our previous study compared the growth of the SVT in chimpanzees and humans during the first two years of life, and showed that—as in humans—chimpanzees show rapid laryngeal descent, with changes in the relative proportion of the SVT_H and SVT_V (Nishimura et al., 2003). Another MRI study (Nishimura, 2005), using a cross-sectional ontogenetic series of embalmed specimens, confirmed this developmental change during early infancy. However, that study also showed that the larynx is lowered only slightly and the horizontal oral cavity grows largely during the juvenile period, causing the proportion of the SVT in chimpanzees to differ from that in humans. Unfortunately, embalming artifacts precluded the study of developmental changes in the position of the hyoid and epiglottis relative to the palate. Therefore, the descent of the chimpanzee larynx is considered to depend primarily on the descent of the laryngeal skeleton relative to the hyoid, but not on the descent of the hyoid *per se* (Nishimura et al., 2003; Nishimura, 2005). Thus, the human 1:1 proportion of the SVT_H to SVT_V is still believed to depend on the greater proportional descent of the hyoid during the evolution of the human lineage (Negus, 1949; P. Lieberman, 1984; Crelin, 1987; Flügel and Rohen, 1991; Houghton, 1993; Laitman and Reidenberg, 1993; Nishimura, 2003, 2005; Nishimura et al., 2003). In fact, the term "descent" of the larynx or hyoid often implies the proportional changes of SVT toward such a configuration (here we use the terms "descent" and "descend" without any such implication). Here, we used MRI to evaluate the developmental changes in SVT anatomy in three living chimpanzees aged between two-and-a-half and five years, and we use the results to discuss the evolution of the descent of the larynx.

Methods

MRI procedures

We have studied the development of three living chimpanzees, named Ayumu (male), Cleo (female), and Pal (female), using MRI. They were born in 2000 and were reared by the biological mothers in the Primate Research Institute (PRI), Kyoto University, Japan (Matsuzawa, 2003). The care and use of the subjects conformed to the guidelines of the PRI (1986, 2002). The chimpanzees were scanned at scheduled intervals from four months to five years of age (Appendix 1). Here, we evaluate the measurements made from two-and-a-half to five years. The scans for Ayumu at four and six months of age were excluded from the analyses in our previous study (Nishimura et al., 2003) because the images were slightly obscured by motion artifacts.

The MRI examination protocol used here was essentially the same as described in a previous study (Nishimura et al., 2003) and was approved by the Ethics Panel of the PRI

(2002). Sagittal tomographic images of the growing chimpanzee head and neck were taken with a General Electrics Signa Profile MRI scanner (0.2 Tesla) at the PRI, using the extremity or head receiving-coil. The subjects were anesthetized intramuscularly with 3.5 mg ketamine hydrochloride (Sankyo Co., Ltd., Tokyo, Japan) and 0.035 mg medetomidine hydrochloride (Meiji Seika Kaisha Ltd., Tokyo, Japan) per kilogram of body weight. After four years of age, they were sedated orally using 3.75 mg of droperidol (in 1.5 ml) before being anesthetized. They were placed supine with their heads fixed to the custom acrylic plastic pad within the coil with belts, so they were always placed in the same posture for scanning. The mid-sagittal plane of the head was adjusted to the laser lights of the MR scanner. This procedure inevitably bends the neck slightly at the level inferior to the vocal folds (Fig. 1). All imaging sequences were sagittal spin echo series with time-to-echo durations ranging from 17 to 32 ms, time-to-repeat durations of 600 ms, fields of view ranging from 18 to 28 cm with 2.7 mm or 3.0 mm slice thicknesses, and 0.8 mm or 0.5 mm gaps between slices using an acquisition matrix of 192 × 192 with two excitations. These parameters were chosen generally based on subject size (Appendix 1). The matrix of all MR images was 256 × 256 pixels, and image resolutions ranged from 0.70 × 0.70 to 1.09 × 1.09 mm/pixel (Appendix 1).

Measurements

MR images were transferred from the scanner to a personal computer, using Vox-Base Transmit software (J-Mac System, Sapporo, Japan). The images were converted from DICOM to TIFF format, using Intage software (KGT, Tokyo, Japan). These images were evaluated using Adobe PhotoShop CS software (Adobe Systems, San Jose, CA, USA) to record measurement points and standard planes. Measurement points and standard planes on the mid-sagittal plane included the following anatomical landmarks: anterior nasal spine (ANS); anterior tubercle of the atlas (ATA); endoprosthion (EPr); hyoid bone (HB); posterior nasal spine (PNS); posterior oropharyngeal wall (POW); palatal plane (PP); posterior pharyngeal wall line (PPW); and vocal fold (VF). These definitions (Table 1) are those defined for chimpanzees by Nishimura (2005) and are roughly equivalent to those used for radiographic studies of humans (D.E. Lieberman et al., 2001). The present study used two additional points: epiglottis (EG) and velum (VL). These definitions (given in Table 1) were those used for chimpanzees by Nishimura et al. (2003).

The coordinate values of the measurement points for linear dimensions were measured three times from MR images,

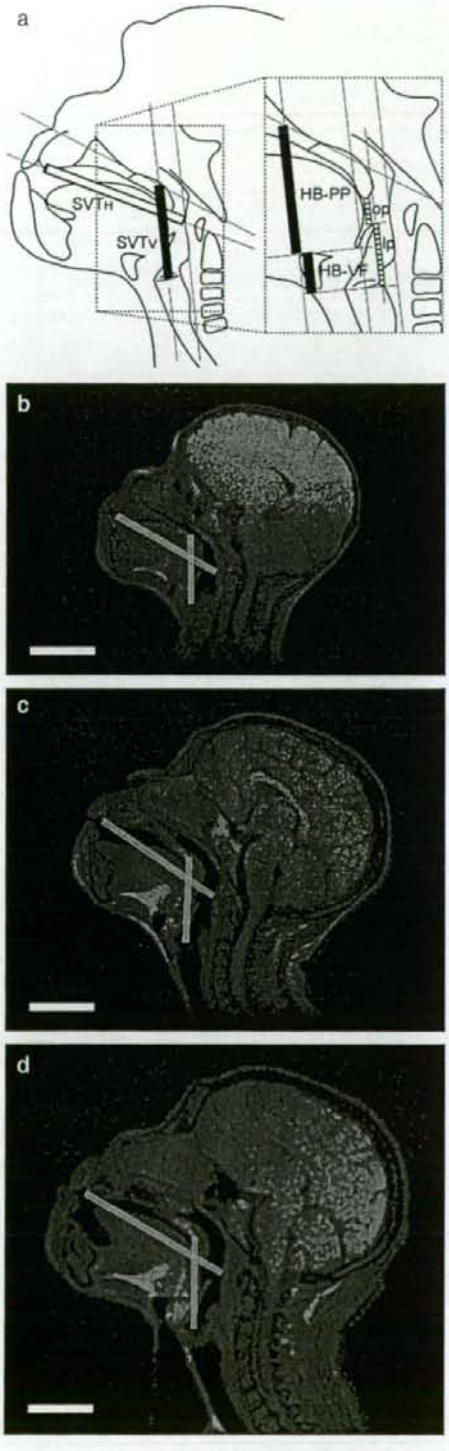

Fig. 1. Growth of the SVT in chimpanzees. a, Left, mid-sagittal diagram of SVT_H (outlined) and SVT_V (filled) lengths. Right, diagram of the distances from the hyoid to the vocal folds (HB–VF) and to the palate (HB–PP); the oropharyngeal (op) and laryngopharyngeal (lp) dimensions. See Methods for definitions of the dimensions. b–d, Mid-sagittal magnetic resonance images of a female chimpanzee (Pal) at: b, four months of age; c, two years of age; and d, four years of age. Scale bar = 3.0 cm.

Table 1
Definitions of the measurement points and standard planes used

Landmarks and planes	Abbr.	Definition
Anterior nasal spine	ANS	The most anterior inferior point of the piriform aperture of the nose, which is roughly equivalent to the most anterior root point of the nasal septum[1]
Anterior tubercle of the atlas	ATA	The superior–inferior midpoint of the anterior tubercle of the atlas[2]
Epiglottis	EG	The most superior point of the epiglottis
Endoprosthion	EPr	The most anterior inferior point of the lingual surface of the alveolar premaxilla
Posterior nasal spine	PNS	The most posterior point of the maxillary body at the level of the nasal floor at the junction of the hard and soft palates
Posterior margin of the oral cavity	POC	Plane from PNS to EPr–POW, parallel to the posterior pharyngeal wall (PPW)
Posterior oropharyngeal wall	POW	Point on the posterior pharyngeal wall opposite the anterior tubercle of the atlas along the plane from EPr to ATA
Palatal plane	PP	The line from the ANS to PNS
Posterior pharyngeal wall	PPW	The most posterior straight line of the pharyngeal wall from the level of the velum to the laryngeal orifice
Vocal folds	VF	The anterior–posterior midpoint of the vocal folds
Velum	VL	The most posterior inferior point of the palatine velum, excluding the uvula

All landmarks and planes were defined on the mid-sagittal plane of the MR images.

[1] This definition is the same as that for chimpanzees used by Nishimura (2005) and Nishimura et al. (2003), but is different from that used by D.E. Lieberman et al. (2001) in the narrow sense because no true anterior nasal spine is found in chimpanzees.

[2] This definition is the same as that used for chimpanzees by Nishimura (2005) and Nishimura et al. (2003), but is different from that used for the human ATA (D.E. Lieberman et al., 2001). The human ATA is defined as the most anterior and superior point of the anterior tubercle of the atlas that projects on the sagittal plane in radiographs. This point was identified only obscurely on mid-sagittal MR images of chimpanzee subjects, so the chimpanzee ATA was here redefined as being roughly equivalent to the human ATA.

using ImageJ software (W. Rasband, National Institute of Mental Health, Bethesda, MD, USA). If the values did not agree, they were remeasured. Measurements included: SVT_H length, along the EPr–ATA line from the EPr to the POW; SVT_V length, parallel to the PPW from the VF to the PP; the distances from the hyoid to the palatal plane and to the vocal folds (HB–PP and HB–VF, respectively), parallel to the PPW from the HB to the PP and to the level of VF (see the keys to Fig. 1a). These definitions of dimensions are those used for chimpanzees by Nishimura (2005) and are similar to those used for humans by D.E. Lieberman et al. (2001). Although there are some inconsistencies in the abbreviations of measurement points and standard planes, the dimensions are the same as those used previously (Nishimura et al., 2003; Nishimura, 2005). The present study also examined the lengths of the oropharyngeal (op) and laryngopharyngeal (lp) parts of the vertical pharyngeal cavity, parallel to the PPW from the EG to the levels of the VL and VF, respectively (see the key to Fig. 1a). These dimensions are the same as those used by Nishimura et al. (2003).

Comparing growth phases between chimpanzees and humans

The measurements were recorded for the chimpanzees at ages ranging from four months to five years (Nishimura et al., 2003; present study). Those for humans by D.E. Lieberman et al. (2001) were taken from one month to thirteen years and nine months of age. The developmental patterns were compared in terms of the developmental ages of both species after their chronological ages were adjusted to correspond to dental eruption stages, which reflect analogous growth phases in both species. In this study, three growth stages, "early infancy", "late infancy", and the "juvenile period", were defined as the dental stage from birth to before the eruption of the deciduous dentition, the stage from the eruption of the deciduous dentition to before the eruption of the first molars, and the stage after the eruption of the first molars and before the eruption of the second molars, respectively. Based on studies of dental eruption ages in captive living chimpanzees (Conroy and Mahoney, 1991; Kuykendall et al., 1992) and European-derived humans (Hurme, 1949; Lysell et al., 1962; Smith and Garn, 1987; Smith, 1991), the chronological ages of the chimpanzees and humans were adjusted to correspond to dental stages. In chimpanzees, "early infancy" is defined here as the period from birth to one year of age, "late infancy" as the period from one to three years of age, and the "juvenile" stage as the period from three to seven years of age. In humans, "early infancy" is defined as the period from birth to two-and-a-half years of age, "late infancy" as the period from two-and-a-half to six years of age, and the "juvenile" stage as the period from six to twelve years of age (Fig. 2). Thus, the developmental patterns were examined here up to five years of age in chimpanzees and compared with those published for up to nine years of age in humans (Fig. 2).

Results

The scan for Ayumu at 52 months of age showed that, at the time of scanning, the tongue was contracted anterosuperiorly, probably causing additional elevation of the hyoid and larynx (Fig. 3; Appendix 2). Although this indicates the dynamic anatomy of the chimpanzee vocal tract (discussed below), the scan was excluded from the analyses of the developmental changes in resting anatomy of the SVT.

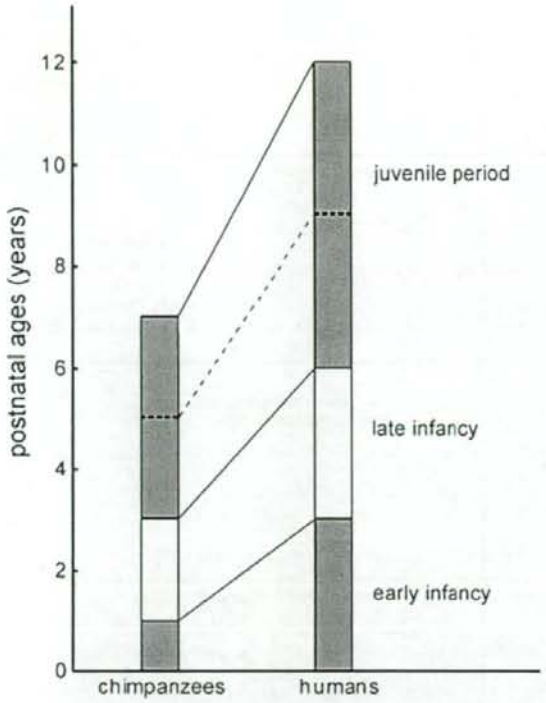

Fig. 2. Analogous growth phases in chimpanzees and humans. The chronological ages of the species are expressed in terms of dental stages, based on the dental eruption ages. Here, we compare the SVT development of both species until the growth phase indicated by the dotted line.

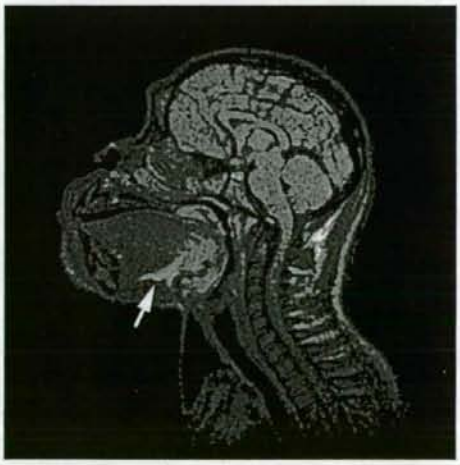

Fig. 3. MRI scan of Ayumu at 52 months of age. When capturing the image the tongue was contracted and the hyoid was pulled antero-superiorly. This is shown by the deformed connective tissue between the *genioglossus* and *geniohyoideus* muscles (arrow; white tissue on the image), compared with the images of Figure 1.

In chimpanzees, the SVT_V increases more than the SVT_H during early infancy (Nishimura et al., 2003), but thereafter the SVT_H shows greater growth relative to that of the SVT_V (Fig. 1). This is reflected in the age-related changes in the ratio of the SVT_H to SVT_V lengths shown in Figure 4a and Appendix 2. Although the absolute values are greater in chimpanzees than in humans, the ratio decreases in chimpanzees—as in humans—during early infancy (Nishimura et al., 2003). After late infancy, it consistently decreases to reach the adult ratio of 1:1 by nine years of age in humans (D.E. Lieberman et al., 2001), whereas in chimpanzees the direction of change in the ratio reverses, and it increases to reach the ratio of 2.10 at five years of age. Figure 4b and Appendix 2 show

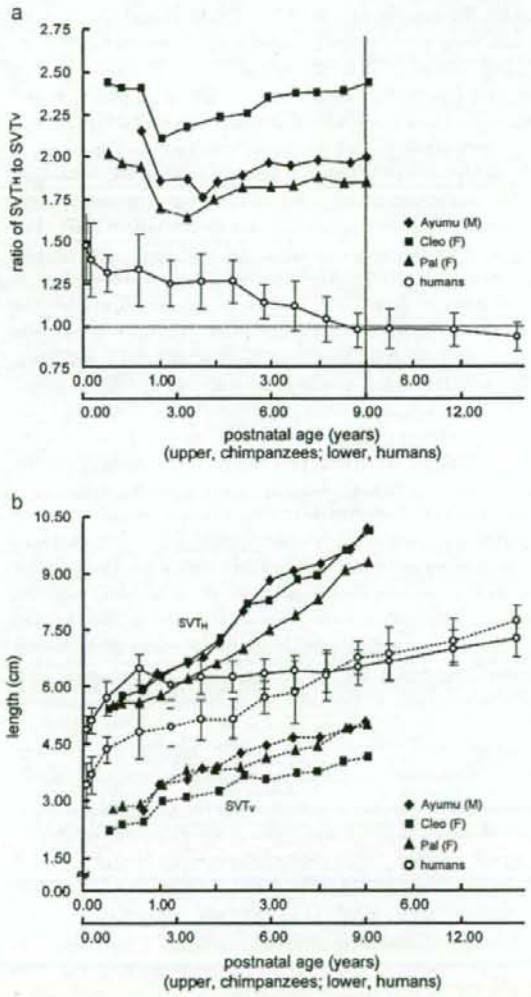

Fig. 4. Growth of the SVT in the three chimpanzees: Ayumu (male, diamonds), Cleo (female, squares), and Pal (female, triangles). Measurements on humans (pooled sexes, open circles) are from D.E. Lieberman et al. (2001), with permission. a, Age-related changes in the ratio of SVT_H to SVT_V lengths. b, Growth of the SVT_H (continuous line) and the SVT_V lengths (dotted line).

the growth changes in SVT_V and SVT_H lengths measured by MRI in living chimpanzees. During early infancy, the growth patterns of these dimensions in chimpanzees are similar to those in humans, although the absolute values of the SVT_V are smaller in chimpanzees than in humans (Nishimura et al., 2003). Thereafter, the SVT_H in humans grows much more slowly (D.E. Lieberman et al., 2001), whereas in chimpanzees it continues to increase greatly and reaches 9.8 cm at five years. In contrast, even after late infancy, the growth pattern of the SVT_V in chimpanzees is similar to that in humans; the chimpanzee SVT_V grew consistently to 4.7 cm at five years of age. Thus, the different changes in the proportions of the SVT between chimpanzees and humans after late infancy do not involve the growth of the SVT_V, and result from the greater growth of the chimpanzee SVT_H.

As for the growth of the SVT_V, the laryngeal skeleton of chimpanzees descends rapidly relative to the hyoid in early infancy, but the hyoid descends only slightly relative to the palate in that period (Nishimura et al., 2003). Thereafter, the laryngeal skeleton continues to descend gradually, but the hyoid descends rapidly. This finding is demonstrated in Figure 5 and Appendix 2. The distance from the hyoid to the vocal fold (HB–VF) increases rapidly to 1.1 cm by the first year of age, whereas the distance from the hyoid to the palate (HB–PP) shows only a negligible change in the first year of life (Nishimura et al., 2003). After late infancy, the HB–VF measure continues to increase to 2.2 cm at five years of age, but the HB–PP increases steeply from 2.9 cm to 5.4 cm at that age. A growth-related change in the descent of the laryngeal skeleton relative to the hyoid, similar to that of chimpanzees, is also observed in humans. Although the human hyoid descends relative to the palate even in early infancy, it continues to descend and this descent is more rapid than the descent of the laryngeal skeleton relative to the hyoid after late infancy (D.E. Lieberman et al., 2001). During that period, the epiglottis continues to descend gradually from the velum in chimpanzees, as in humans, to lengthen the oropharyngeal part rostral to the laryngopharyngeal parts (Fig. 5c, Appendix 2). Thus, after late infancy in chimpanzees, the major descent of the larynx is also accounted for by the descent of the hyoid, as in humans, and it causes elongation of an oropharyngeal part.

Discussion

This study provides no evidence for the idea that the rapid descent of the larynx or hyoid arose uniquely in the human lineage to allow the configuration of the equally long SVT_H and SVT_V to develop and thus permit the acquisition of speech in this lineage (Negus, 1949; P. Lieberman, 1984; Crelin, 1987; Houghton, 1993; Laitman and Reidenberg, 1993; Nishimura, 2003, 2005; Nishimura et al., 2003). Some gross anatomical studies using a cross-sectional ontogenetic series of embalmed chimpanzee specimens have suggested a developmental lowering in the position of the larynx or hyoid in chimpanzees (Kelemen, 1948; Avril, 1963; Jordan, 1971), but it was unclear whether this was analogous to the descent of the larynx in

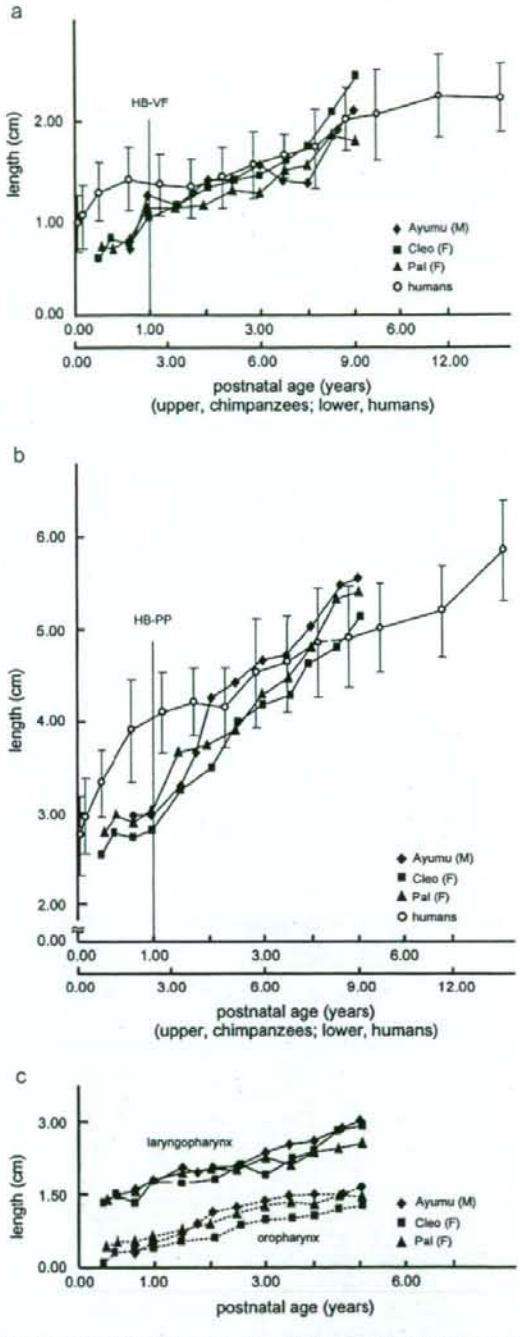

Fig. 5. Increases in the dimensions of the SVT_V. a, Distance from the hyoid to the vocal folds (HB–VF). b, Distance from the hyoid to the palate (HB–PP). c, Growth of the laryngopharyngeal (lp, continuous line) and oropharyngeal (op, dotted line) parts of the vertical pharyngeal cavity. The symbols for individual subjects used in the present study and for humans taken from D.E. Lieberman et al. (2001) are as in Figure 4.

humans. Our previous study (Nishimura et al., 2003) confirmed that chimpanzees show a laryngeal descent with changes in the relative proportion of the SVT_H to SVT_V during early infancy, but this is not identical to the phenomenon in humans. Another MRI study using a cross-sectional ontogenetic series of embalmed chimpanzee specimens (Nishimura, 2005) showed relative changes in the SVT_H and SVT_V similar to those observed in the present study. However, the low resolution of the cross-sectional samples allowed the conclusion to be drawn that the adult configuration of the chimpanzee SVT results, at least partly, from a lesser descent of the larynx compared with that in humans, i.e., a lack of any major descent of the hyoid (Nishimura, 2005). Here, our longitudinal MRI study confirms that this descent of the hyoid is shared with chimpanzees, despite differences in the developmental trajectory of the SVT configuration between humans and chimpanzees.

However, there are slight differences in the timing and rate of the hyoid descent in humans and chimpanzees. Although this issue must be resolved in the future using a larger sample, it might be caused by the development of the laryngeal air sac in chimpanzees. Whereas humans have no sac, chimpanzees have a huge sac extending superiorly from the bilateral laryngeal ventricles, between the hyoid and thyroid cartilage bilaterally, and expanding anteriorly to the ventral region of the neck (Kelemen, 1948; Negus, 1949; Starck and Schneider, 1960; Avril, 1963; Hayama, 1970; Hewitt et al., 2002). This sac developed to reach the dorsal aspect of the body of the hyoid at four months of age for all three subjects in the present study (a small dark pouch on Fig. 1b; Nishimura et al., in press), and the associated anatomical changes might thereby contribute to elevate the hyoid in early infancy.

Regardless of these slight differences, the descent of the chimpanzee larynx and hyoid continues to lower the epiglottis from the velum and pulls the tongue down in the pharynx, elongating an oropharyngeal region facing the dorsal surface of the tongue. The chimpanzee epiglottis is believed to maintain contact with or to remain close to the velum even in adults, based on examinations using embalmed cadavers (Laitman and Heimbuch, 1982; P. Lieberman, 1984; Fitch, 2000a; Nishimura, 2005). However, MRI studies in living chimpanzees showed that the epiglottis loses contact with the velum in early infancy (Nishimura et al., 2003), as in humans (Sasaki et al., 1977), so that an oropharyngeal region is secured in the adult (Nishimura, 2006). Inevitably, the position of the epiglottis relative to the velum is affected by embalming artifacts (Nishimura, 2005) and varies actively in living animals (see Fig. 3; Fitch, 2000b). The latter suggests that the head posture at scanning (Nishimura et al., 2003; Nishimura, 2006; present study) might always lower or additionally elevate the epiglottis of the living subjects, although the present analysis carefully excluded any measurements in the nonresting subject (Ayumu at 52 months of age). However, in these studies, the neck of all the subjects was always bent slightly at the level below the vocal folds (Nishimura et al., 2003; Nishimura, 2006; present study). In addition, a varied head posture has little influence on the SVT_V dimension, which reflects the position of the vocal folds relative to the palatal plane (Nishimura, 2005). This suggests that the position of the epiglottis, which is attached to the thyroid cartilage of the laryngeal skeleton, is also retained relative to the velum, regardless of the varied head posture. These facts suggest that varying head postures have limited influence on the over- or underestimation of oropharyngeal measures. This study showed the steady elongation of the oropharyngeal region, under the same conditions; unfortunately, there is no comparable information about the growth rates of the human oropharyngeal and laryngopharyngeal components. On the other hand, the human pharynx, defined as a linear dimension from VL to VF, increases from 2.63 cm at two to four years of age to 3.92 cm at nine to ten years of age, an increase of 49% (Fitch and Giedd, 1999). This increase is similar to the increases in sum of the oropharyngeal and laryngopharyngeal parts in the corresponding periods in chimpanzees: the sum of the two parts increases from 2.84 cm at two years of age to 4.30 cm at age five, an increase of 51% (Appendix 2). In humans, the pharyngeal dimension continues to increase to 5.64 cm in the adult (19 to 25 years of age), an increase of 114%. Despite little comparable information in living chimpanzees, an adult male subject at 23 years of age shows the sum of 5.58 cm, an increase of 96% (unpublished data, although the MR image is shown in Nishimura, 2006). Taking consideration of the secondary descent of the human larynx in the pubertal period (an additional elongation of the pharyngeal dimension; Fitch and Giedd, 1999), this indicates that the development of the pharyngeal dimension is similar in the two species. In addition, the timing in the loss of contact between the epiglottis and velum is similar approximately analogous in humans and chimpanzees: before 12 to 18 months in humans (Sasaki et al., 1977), and before 6 months in chimpanzees (Nishimura et al., 2003). This suggests that the development of the oropharyngeal dimension in chimpanzees is approximately analogous to that in humans. Although this issue must be resolved in the future using larger samples of humans and chimpanzees with varied head positions, it is probable that the major descent of the larynx, elongating an oropharyngeal region, was shared fully by the last common ancestor of chimpanzees and humans.

Uncertainty remains regarding the evolutionary path involving the major descent of the laryngeal skeleton and hyoid before the divergence of the human from the chimpanzee lineage. A study using computed tomography (CT) demonstrated a slight descent of the larynx and hyoid relative to the palate in macaques from birth to 24 years of age (Flügel and Rohen, 1991). The measurements reported by those authors cannot be directly compared with the studies of humans (D.E. Lieberman et al., 2001) and chimpanzees (Nishimura et al., 2003; Nishimura, 2005; present study) because of a discrepancy in the definitions. However, they suggest that an increase of the vertical dimension of the SVT in the first six months of macaques may be roughly similar to those in the corresponding periods of the other two species, although the measurements after six months of macaques must be excluded because of their

scarcity and irregularity. Based on the studies of dental eruption ages in macaques (Schultz, 1933; Iwamoto et al., 1984), the period from birth to six months of age corresponds to "early infancy" defined here for humans and chimpanzees. In macaques, the distance from the palate to the glottis increases from 1.2 cm on day one to 1.9 cm at six months of age, an increase of 58% (Flügel and Rohen, 1991). The SVT_V in humans increases from 3.40 cm at one month to 4.92 cm at two years and nine months of age, an increase of 44% (D.E. Lieberman et al., 2001). In chimpanzees, it grows from 2.47 cm at four months to 3.26 cm at one year of age, an increase of 32% (Nishimura et al., 2003; see Appendix 2). Taking consideration of a lack of measurements in the periods earlier than one month for humans and four months for chimpanzees, these increases in the vertical dimension of the SVT in early infancy are similar in all three species. However, it must be noted that macaques have a laryngeal air sac opening above the anterior commissure of the glottis, extending superiorly to reach the dorsal aspect of the hyoid body and expanding inferiorly to the ventral region of the neck (Negus, 1949; Hayama, 1970; Hewitt et al., 2002). Although the development of this macaque air sac is still unclear, the developing sac might lower the laryngeal skeleton additionally relative to the hyoid in early infancy. In fact, although the descent of the larynx also continues after late infancy in macaques, CT scans show that the descent in macaques does not separate the epiglottis from the velum, producing no or little oropharyngeal space even in adults (Flügel and Rohen, 1991). Moreover, the laryngeal skeleton is separated from the hyoid and assured of mobility independent of the hyoid in hominoids, including humans, but this is in contrast to other anthropoids whose laryngeal skeletons are locked into the hyoid body and are tied tightly with or directly articulated with the greater horn of the hyoid, according to the comparative anatomy of adult cadaver specimens (Nishimura, 2003). Although this issue must be resolved in the future using larger samples and similar methods, these facts suggest that the descent of the larynx in macaques is not analogous to that seen in humans and chimpanzees. The major descent of the larynx may thus have evolved in two steps: the descent of the laryngeal skeleton relative to the hyoid in a common ancestor of all extant hominoids (Nishimura, 2003; Nishimura et al., 2003), and descent of the hyoid relative to the palate in a common ancestor of humans and chimpanzees. On the other hand, the descent of the laryngeal skeleton may have accompanied the descent of the hyoid. The hyoid provides the base of the tongue muscles and it is physically linked to the laryngeal skeleton, mandible, and cranial base through muscles, ligaments, and other soft tissues (Zemlin, 1988; Titze, 1994). The hyolaryngeal complex plays an important role in integrated functions of the pharynx and larynx, such as deglutition, breathing, and vocalization (Negus, 1949; P. Lieberman, 1984; Crelin, 1987; Zemlin, 1988; Hiiemae and Palmer, 1999). The complexity of the anatomy and the integrated nature of the vital functions of the hyolaryngeal complex raise the possibility that the major descent of the laryngeal skeleton and hyoid may have arisen in a single shift to facilitate these integrated functions in a common ancestor of extant hominoids.

It is likely that the major descent of the larynx originally conferred advantages for functions unrelated to the sophisticated articulation of speech, because the faculty arose in the human lineage after its divergence from the chimpanzee lineage. This argument is also supported by the situation of a descended larynx in nonprimate mammals (Fitch, 2000b; Fitch and Reby, 2001; Weissengruber et al., 2002; Reby et al., 2005). Fitch and Reby (2001) showed that the position of the larynx in the males of some species of deer is similar to that in adult humans and that they lower the larynx to produce resonance at lower frequencies during the roaring of the rut season. This modification in vocalization possibly contributes to exaggeration of their perceived body size to increase the repulsive effects against other males (Reby et al., 2005). The ability to lower the hyolaryngeal complex is also found in the vocalizing of dogs, which have a resting anatomy with the epiglottis maintaining contact with the velum (Fitch, 2000b). That such dynamic lowering of the larynx exists in independent lineages clearly indicates the selective advantage or advantages of vocalization, independent of speech and language, and can account for the static low position of the larynx that was achieved with the developmental descent of the larynx in humans and chimpanzees. Such nonlinguistic advantage might be achieved by the secondary descent of the human larynx, which effects formant frequencies and probably underlies the vocal remodeling process during the puberty of human males (Fitch and Giedd, 1999). However, the integrated nature of the function of the hyolaryngeal complex makes it unlikely that any single selective advantage involving vocalization can account for the major descent and resulting low position of the larynx. The integration of deglutition and breathing suggests that an advantage in such physiology unrelated to vocalization may partly account for it (Nishimura, 2003). Such dynamic capabilities of the vocal tract and hyolaryngeal complex strongly indicate that critical evidence for this issue will be provided by evaluations of dynamic activities to perform a physiological function, not just by evaluations of the static anatomy of the SVT. Chimpanzees also show some dynamic activities, for example contraction of the tongue to pull up the hyoid (see Fig. 3) and retraction of the tongue to lower the hyolaryngeal complex (see Fig. 3 in Nishimura, 2005); unfortunately, there is no information about the relationships between such activities and physiological performances. Thus, such information in nonhuman primates and mammals will facilitate our understanding of the evolutionary pathway involving the major descent and static low position of the larynx against both phylogenetic and functional backgrounds.

Although this study suggested that the descent of the larynx and hyoid in chimpanzees is analogous to that in humans, but not to that in macaques, the adult configuration of the SVT in chimpanzees resembles that of macaques

rather more than humans (Nishimura, 2005, 2006). Here, the ratio of SVT_H and SVT_V lengths in chimpanzees is higher than that in humans already in infancy, and it increases after late infancy—in contrast to humans—to result in a distinct final SVT configuration. Such differences between humans and chimpanzees were probably established by the evolution of facial flattening—the reductions of facial prognathism and projection—in the human lineage, but not by that of laryngeal descent itself. Similar developments in the pharyngeal configuration strongly suggest that the SVT_V dimension in chimpanzees probably corresponds approximately to that in humans even at birth and later, despite smaller absolute values of the SVT_V length in chimpanzees than in humans. This is supported in particular by the similar timing of the loss of contact between the epiglottis and velum. In contrast, the MR images show that the face is already projected and prognathic in chimpanzee infants (Fig. 1b). These characteristic differences in facial shape between the two species partly arise in prenatal development (Enlow, 1990; Ackermann and Krovitz, 2002; Penin et al., 2002; Mitteroecker et al., 2004). These facts suggest that the SVT_H dimension in chimpanzees does not correspond to that in humans already at birth, although the absolute values of the SVT_H length per se are similar. If so, this difference is principally involved in causing the higher ratio of the SVT_H and SVT_V lengths during infancy in chimpanzees compared with humans. In addition, after late infancy, the disparity of the SVT_H growth trajectory contributes to differentiate further the SVT configuration in chimpanzees from that in humans. This fact supports the idea that the evolutionary attainment of a 1:1 proportion of the SVT_H and SVT_V required facial flattening rather than the additional descent of the larynx in the human lineage. Such reorganization of the facial architecture probably involved modifications to mandibular form. In anatomical terms, modification of the mandibular height affects the position of the hyoid (D.E. Lieberman et al., 2001), and therefore the facial flattening might have been accompanied by an additional descent of the hyoid. However, this does not mean that such facial flattening squeezed the tongue into the pharynx, which makes the larynx descend along the neck (Negus, 1949; Flügel and Rohen, 1991; Aiello, 1996, 1998; Mithen, 2005). The present study provides critical evidence for the idea that facial flattening merely produced a short, horizontal dimension for the tongue, fitting the SVT_H configuration in the human lineage, independently of the descent of the hyoid, which had already increased the vertical dimension. These reductions in facial prognathism and projection were long-term trends in primate and human evolution (Klein, 1989; Wood, 1992; Aiello and Dean, 1998; Fleagle, 1999; Wood and Collard, 1999; Trinkaus, 2003), and therefore it is unlikely that the selective advantage afforded by speech originally accounted for this rearrangement. Thus, facial flattening has probably evolved only secondarily, with anatomical consequences that facilitated the evolution of sophisticated spoken language in the human lineage.

Conclusions

The major descent of the larynx and the reduced growth of the face essentially constitute the "ontogeny" of the morphological foundations of speech in humans (Negus, 1949; P. Lieberman, 1984; Crelin, 1987; Zemlin, 1988; Flügel and Rohen, 1991; Laitman and Reidenberg, 1993; Fitch and Giedd, 1999; D.E. Lieberman et al., 2001). However, this study does not support the "evolutionary" hypothesis that this descent occurred in the human lineage, and thereby produced the SVT configuration with an equally long SVT_H and SVT_V, leading to the origins of speech. The major descent of the larynx arose before the diversification of human and chimpanzee lineages, but it has not led to such a configuration of the SVT. Briefly, the term "descent" of the larynx has no implication for the proportional changes toward such an SVT configuration. The attainment of the SVT configuration unique to humans required facial flattening—modifications in the facial development to reduce the oral horizontal dimension—rather than additional laryngeal descent in the human lineage. These two separate developmental mechanisms probably arose independently during the evolution of nonhuman primates and humans, which were secondarily advantageous for speech in the latter. Therefore, it is unlikely that the faculty for speech evolved in humans through a single coordinated adaptive shift in all the relevant biological foundations. Instead, we propose the concept of a multiple-step evolution of the disparate elements in response to different selection pressures during the long period of primate evolution. This is also possibly the case for the evolution of the cognitive foundations of language (e.g., Matsuzawa, 2001). Rather than using one unique feature as evidence for the evolution of speech and language, a multidisciplinary approach should contribute greatly to our understanding of the evolution of the biological foundations of language.

Acknowledgments

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Appendix 1. Subjects used, their ages at the times of scans, and major parameters of scanning

Subjects	Age (months)	Pixel sizes[1] (mm/pixel)	FOV (mm)	Thickness (inter-slice gap)[2] (mm)
Ayumu	4	0.74	190	2.7 (0.8)
	6	0.70	180	3.0 (0.5)
	9	0.74	190	2.7 (0.8)
	12	0.70	180	3.0 (0.5)
	18	0.70	180	3.0 (0.5)
	22	0.90	230	2.7 (0.8)
	25	0.86	220	3.0 (0.5)
	30	0.98	250	3.0 (0.5)
	36	1.09	280	3.0 (0.5)
	42	0.98	250	3.0 (0.5)
	48	0.98	250	3.0 (0.5)
	52	0.98	250	3.0 (0.5)
	54	0.98	250	2.7 (0.8)
	60	0.98	250	3.0 (0.5)
Cleo	4	0.70	180	3.0 (0.5)
	6	0.70	180	3.0 (0.5)
	9	0.70	180	3.0 (0.5)
	12	0.74	190	2.7 (0.8)
	18	0.70	180	3.0 (0.5)
	25	0.86	220	3.0 (0.5)
	31	0.98	250	3.0 (0.5)
	36	0.98	250	3.0 (0.5)
	43	0.98	250	3.0 (0.5)
	48	0.98	250	3.0 (0.5)
	54	0.98	250	3.0 (0.5)
	60	0.98	250	3.0 (0.5)
Pal	4	0.70	180	3.0 (0.5)
	6	0.74	190	2.7 (0.8)
	9	0.70	180	3.0 (0.5)
	12	0.70	180	3.0 (0.5)
	18	0.70	180	3.0 (0.5)
	24	0.86	220	3.0 (0.5)
	30	0.98	250	3.0 (0.5)
	36	0.98	250	3.0 (0.5)
	42	0.98	250	3.0 (0.5)
	48	0.98	250	3.0 (0.5)
	54	0.98	250	3.0 (0.5)
	60	0.98	250	3.0 (0.5)

[1] The values varied irregularly during infancy, but had little influence on the accuracy in measurements (see Results).

[2] This means that the slice interval of all scans was 3.5 mm, regardless of differences in slice thickness and inter-slice gap.

Appendix 2. Measurements of dimensions

Subjects	Age (months)	SVT_H	SVT_V	Oropharynx (op)	Laryngopharynx (lp)	HB–PP	HB–VF
Ayumu	9	5.93	2.75	0.29	1.60	3.00	0.70
	12	6.33	3.41	0.49	1.78	2.98	1.27
	18	6.59	3.53	0.67	2.07	3.39	1.14
	22	6.78	3.84	0.89	1.97	3.71	1.28
	25	7.17	3.87	1.12	2.05	4.30	1.41
	30	8.03	4.25	1.23	2.08	4.46	1.42
	36	8.80	4.47	1.35	2.38	4.68	1.58
	42	9.04	4.65	1.45	2.54	4.74	1.42
	48	9.25	4.67	1.48	2.61	5.06	1.38
	52	9.06	4.63	1.11	2.85	4.17	1.61
	54	9.63	4.90	1.48	2.86	5.50	1.95
	60	10.16	5.08	1.66	3.03	5.57	2.19
Cleo	4	5.39	2.21	0.07	1.31	2.57	0.60
	6	5.73	2.39	0.30	1.50	2.81	0.80
	9	5.88	2.45	0.34	1.30	2.76	0.76
	12	6.26	2.97	0.39	1.78	2.83	1.03
	18	6.66	3.06	0.53	1.71	3.34	1.16
	25	7.27	3.26	0.60	1.82	3.58	1.32
	31	8.19	3.64	0.87	2.11	4.04	1.41
	36	8.29	3.53	0.97	1.91	4.22	1.47
	43	8.84	3.73	1.00	2.24	4.31	1.62
	48	8.95	3.76	1.06	2.43	4.69	1.78
	54	9.59	4.02	1.17	2.82	4.85	2.19
	60	10.10	4.15	1.25	2.93	5.14	2.58
Pal	4	5.50	2.73	0.42	1.39	2.80	0.72
	6	5.58	2.85	0.52	1.48	2.99	0.70
	9	5.56	2.87	0.53	1.57	2.91	0.82
	12	5.78	3.41	0.63	1.81	3.04	1.12
	18	6.22	3.80	0.75	1.97	3.73	1.13
	24	6.60	3.79	0.88	2.04	3.81	1.16
	30	7.01	3.85	1.10	2.01	3.97	1.31
	36	7.50	4.12	1.26	2.28	4.32	1.28
	42	7.88	4.31	1.33	2.11	4.50	1.52
	48	8.31	4.41	1.27	2.38	4.81	1.57
	54	9.04	4.90	1.47	2.46	5.29	1.92
	60	9.25	5.00	1.47	2.56	5.39	1.82

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Origins of smile and laughter: A preliminary study

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KEYWORDS
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Abstract To present fundamental data, spontaneous smiles and spontaneous laughs (smiles accompanied by vocal sounds) were cross-sectionally observed in 10 newborn infants and longitudinally observed in six infants. Unilateral spontaneous smiles were more common than bilateral smiles in neonates, but by 2 months almost all spontaneous smiles were bilateral. All spontaneous laughs were bilateral. "Spontaneous smile" and "Spontaneous laugh" might be different behaviors from the beginning.
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Human newborn infants show spontaneous smiling, "a slow, gentle, sideward and upward pull of mouth, without rhythmical movements or contraction of other facial muscles," after the first 24 h during irregular sleep, drowsiness, and alert inactivity [1]. This smile occurs without known external or systematically demonstrable internal causes [2].

Although several researchers have observed spontaneous smiles [3–6], except for the work of Shimada [7] and Messinger et al. [8] there has been little systematic research. Shimada studied the phenomenon in 84 newborns from 1 to 7 weeks of age and observed three types of spontaneous smile: incomplete smile, half smile (unilateral, see Fig. 1), and complete/full smile. He found that spontaneous smiles tend to be more frequent at first, and, with time, their frequency decreases while their duration

Figure 1 Unilateral spontaneous smile.

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