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Vocal communication in frogs

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The robust nature of vocal communication in frogs has long attracted the attention of natural philosophers and their biologically inclined successors. Each frog species produces distinctive calls that facilitate pre-mating reproductive isolation and thus speciation. In many terrestrial species, a chorus of simultaneously calling males attracts females to breeding sites; reproductive females then choose and locate one male, using distinctive acoustic cues. Males compete with each other vocally and sometimes physically as well. Anuran acoustic signaling systems are thus subject to the strong pressures of sexual selection. We are beginning to understand the ways in which vocal signals are produced and decoded by the nervous system and the roles of neurally active hormones in both processes.

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Abbreviations

AVT	arginine vasotocin
CNS	central nervous system
ER	estrogen receptor
PRR	pulse repetition rate

Introduction: vocal signals

Anurans (frogs and toads) use acoustic signals during reproduction to convey information on reproductive state and on territorial boundaries. Some of the most intensively studied North American species have been the leopard frog and the bullfrog [1] as well as various treefrogs [2]. The túngara frog from Central America is another widely studied terrestrial model system [3]. In this species the addition of 'chucks' to call whines increases the attractiveness of the male's advertisement call to females at the cost of attracting bat predators [4]. Whines descend in frequency; chucks are briefer bursts of non-frequency modulated sounds. Although vocal advertisement is usually a male characteristic, some females, including those of the aquatic African species *Xenopus laevis*, can advertise their fertility or pending oviposition [5]. In response to a clasp attempt by a male, both sexes can signal a non-reproductive state using release calls. Males compete vocally at boundaries between territories using encounter or aggressive calls [6]. The vocal repertoire of frogs thus typically consists of advertisement and aggressive calls (usually given by males) and release calls (usually given by females). This review focuses on recent themes of interest to neurobiologists: how acoustic information is combined with visual information to increase the salience of the communication signal, how temporal aspects of these acoustic signals are produced and decoded and how endocrine signals and neuromodulators interact within the nervous system during vocal signaling.

Binding: combining vocal and visual signals

In many frogs, vocal advertisement is accompanied by the inflation of a visually obvious vocal sac. Recent evidence indicates that a pulsating sac increases the attractiveness of advertisement calls in túngaras [7••]. Females were tested with whine–chucks broadcast from speakers placed above LCD monitors displaying a video of a túngara male (vocal sac inflated or not) or a synthetic 'rectangle' animation designed to mimic the color, amplitude and frequency of the horizontal movement of the vocal sac. The inflated sac substantially increased the attractiveness of the call to the female over the uninflated sac, whereas the rectangle animation failed to increase call attractiveness. Conspicuous vocal sac inflation coordinated with calling might help females find males in dense choruses. It is even possible that this function, rather than acoustic modulation, accounts for the appearance of conspicuous sacs in so many frogs.

The increased salience provided by visual signals also applies to male–male aggressive signaling. Narins *et al.* [8••] have managed to induce male poison dart frogs (Dendrobatidae) to actually attack an electromechanical frog as long as 'he' is calling and 'his' vocal sac is inflated (Figure 1). This preparation has allowed the group to attack a classical problem in neuroscience, the binding problem. With regards to visual and auditory stimuli binding is embodied in the McGurk effect (see http://www.cee.hw.ac.uk/~cmj/projects/McGurk/download.html) [9], in which the lips of a talking head produce one sound while the audio track plays a second; the receiver hears a sound intermediate between visual and auditory signals. Narins and co-workers have now separated auditory and visual signals from the electromechanical frog in time and space and report that a full response requires temporal overlap and a spatial sound source separation less than or equal to 12 cm [10].

Figure 1

A male poison dart frog (*Epipedobates femoralis;* on the right) attacking the electromechanical frog (on the left). Note that the robotic frog's vocal sac is inflated. This feature must be synchronized with calls broadcast from the speaker on the left to evoke an actual attack. Used with permission from [8••].

Vocal dominance

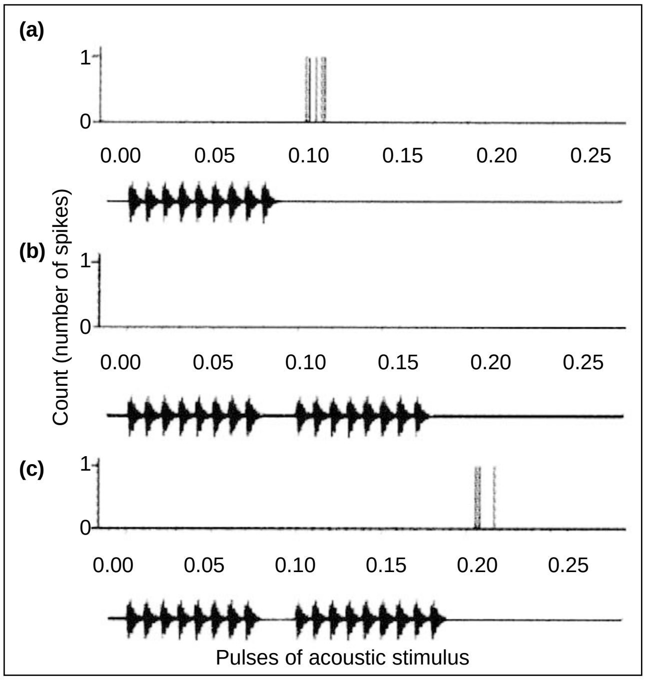
Deafening choruses of simultaneously advertising males pose major hurdles (spatial masking) for a female trying to locate a particular male by his song. In terrestrial frogs, however, the diminution of sound with distance provides some assistance. Localization can also be assisted by visual signals (see above) and involves inhibitory neural circuitry (see below). The story is quite different for frogs that communicate under water, specifically clawed frogs, which live in murky ponds and call only at night. There is no evidence that visual cues are used underwater; instead frogs rely entirely on acoustic signaling (perhaps aided by their lateral line sensory system) and the vocal repertoire can be particularly rich. In the most intensively studied species, *X. laevis*, males produce six distinct calls and females two; these include the advertisement and release calls common to most frogs but also a female 'acoustic aphrodisiac' call, and several male–male call types [11]. The puzzling observation that, unlike terrestrial frogs, only one or two males in a pond advertise at any one time, has recently been clarified by laboratory studies demonstrating robust male–male vocal suppression [12•]. If allowed to physically interact, males clasp each other and engage in vocal duets that use many male specific calls; one male is rapidly silenced. The clasping male is usually vocally dominant but dominance can be established entirely without physical contact. Differences between *X. laevis* and other frogs are probably attributable to the lack of diminution of acoustic signal strength under water; whether or not the vocally dominant frog is more reproductively successful than his silent pond mates remains to be determined.

The perception of vocal signals

How are vocal signals decoded? The classical work of Capranica and co-workers [13] focused attention on the sound frequency domain, especially on the role of the amphibian papilla in detecting low frequency sounds and the basilar papilla in detection of higher frequency sounds. Recent studies have moved to investigating coding in the temporal domain (pulse or click repetition rate, rate of amplitude modulation), because this feature serves to distinguish calls of sympatric species [13] as well as functionally different calls within a species [14,15]. The frog auditory system closely resembles the general vertebrate plan. Auditory information enters the hindbrain through the eighth nerve. Neurons in the dorsal acoustic medulla (cochlear nuclei) and superior olive project to the major auditory midbrain nucleus, the torus semicircularis (inferior colliculus), which projects in turn to auditory thalamus.

Where and how are temporal features coded in the frog central nervous system (CNS)? Rose and his co-workers [16] have recently made a series of striking discoveries on temporal coding using the Pacific tree frog, a species in which pulse repetition rate (PRR) distinguishes male aggressive calls from advertisement calls. Neurons in the auditory midbrain behave as though they have been subjected to temporal filtering. Four kinds of rate-sensitive neurons have been described: low-pass, high-pass, band-pass and band-suppression [17]. Band-suppression neurons respond to high and low PRRs [18••] but not to mid-range rates. Band-pass neurons that are integration units do not respond to the aggressive calls [17]. Manipulation of pulse amplitude revealed

Figure 2



Neurons that count. Neurons in the torus semicircularis of the Pacific treefrog (*Hyla regilla*) require stringent temporal characteristics of an acoustic stimulus to produce spike trains. **(a)** An effective train of 9 pulses, **(b)** two ineffective trains of 8 pulses and **(c)** a train of 8 pulses followed by a train of 9 pulses; only the latter is effective. The inter-pulse interval was 10 ms and the inter-train interval was 20 ms. Used with permission from [19].

that rate *per se* rather than stimulus intensity was being integrated neuronally. Rate-tuned neurons can count pulses [19]. For example, a rate-tuned neuron that requires 9 pulses separated by 10ms to fire (Figure 2) can be completely reset by an interval of 20ms; these longer interpulse intervals are characteristic of aggressive calls. Neurons that are very sharply tuned to a specific PRR ('narrow-band') can be reset by an interval differing by only ~2 ms from the optimal. What is the mechanism for counting? Intracellular recording reveals that the first pulse produces a profound hyperpolarization in rate-tuned toral neurons and subsequent sound pulses produce depolarizations. 'Counting' involves using optimal interpulse intervals and excitatory post-synaptic potentials that increase progressively in size to bring the neuron to threshold for firing an action potential [20].

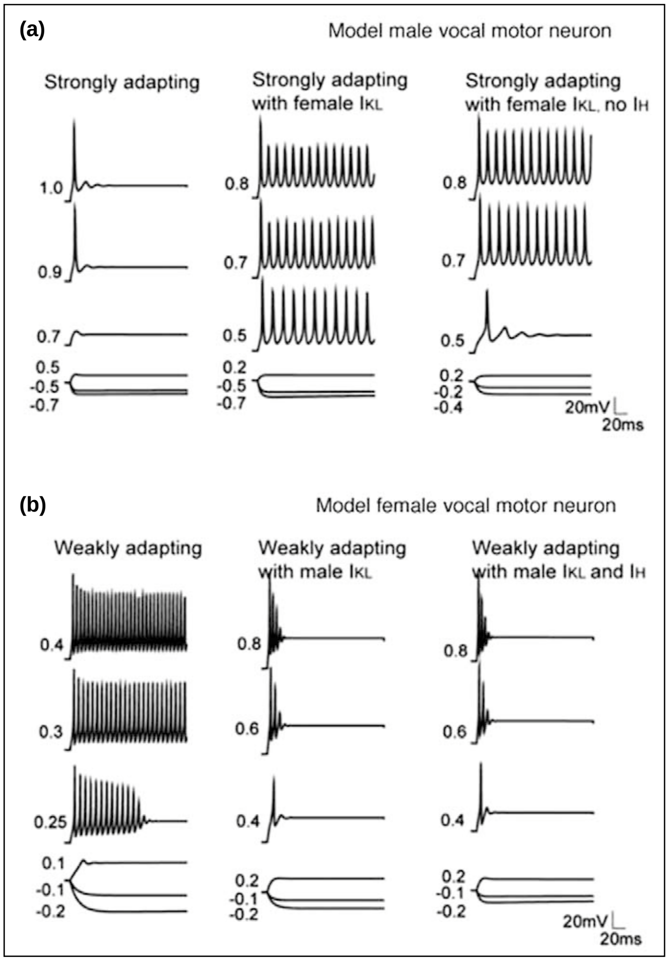
Feng and co-workers [21•] have tackled the problem of spatial masking described above (Vocal dominance paragraph) in the torus semicircularis. The spatial unmasking ability of single toral neurons was abolished by treatment with bicuculline, a γ-amino butyric acid (GABA) receptor antagonist; the unmasking ability of treated neurons was reduced to the level seen in the auditory nerve (i.e. very large spatial separation of sound sources was required). In many neurons, the effect could be attributed to interference with binaural processing that is involved in directional coding. The actual inhibitory circuitry involved has not yet been identified.

Accurate sound localization underlies the ability of gravid females to choose an individual male within a chorus. Is the torus dedicated to this task or are higher centers (thalamus, forebrain) required? Frishkopf *et al.* [22] originally emphasized the role of the posterior and central thalamic auditory nuclei in 'mating call detection' because, among other factors, it is at this level that inputs from the amphibian and basilar papillae converge. The central thalamus also has units tuned to specific rates of amplitude modulation [23]. The very long refractory periods shown by thalamic neurons following acoustic stimulation [24], however, argue against a role in phonotaxis, as does the recent observation in treefrogs that toral, but not thalamic, lesions abolish female phonotaxis [25•]. The connections between auditory thalamus and torus are reciprocal (stimulation of the thalamus, for example, can alter acoustic responses of toral neurons; [26]). Given this feature, and limitations of interpretation of lesion studies, a modulatory role for thalamus might yet be found. In the meantime, however, speculation on the function of thalamic nuclei focuses on attention and long-term endocrine modulation (see below).

The production of vocal signals

How are vocal signals produced? Calls are encoded by activity in the laryngeal and glottal nerves (for terrestrial frogs this activity is coordinated with actual respiration). The requirements of underwater sound production in *X. laevis* have uncoupled song from actual breathing and facilitated analysis of vocal patterns. In singing frogs, the pattern of laryngeal nerve activity matches the temporal patterns of each vocalization; there is a 1:1 correspondence of each click, nerve compound action potential and muscle compound action potential [27]. In a slice preparation of the vocal centers in the *X. laevis* hindbrain [28••], two distinct firing patterns are found in different proportions in males and females. The strongly adapting neurons that predominate in males initiate spikes at short reliable latencies, whereas the weakly adapting motor neurons characteristic of females translate graded levels of depolarization into different firing rates. Low-threshold potassium currents predominate in males. Hyperpolarization-activated cationic currents are found almost exclusively in males. Modeling indicates that sex-typical active and passive properties can account for the sex-typical occurrence of strongly and weakly adapting spike trains (Figure 3). These physiological differences facilitate transformation of synaptic inputs into male- and female-specific outputs that generate sexually distinct songs *in vivo*. Because the motor neurons convey and

Figure 3



Female-typical currents can feminize a model male vocal motor neuron **(a)** and male-typical currents can masculinize a model female vocal motor neuron **(b)**. Strong adaptation facilitates the precise spike timing that underlies rapid stereotyped male advertisement calls in *Xenopus laevis*, clawed frogs. Weak adaptation is matched to female vocal demands; female calls are slower and more variable. Used with permission from [28••].

transform, but do not create, temporally patterned output, attention has turned to how vocal patterns are generated by interneuronal circuitry.

Endocrine and neuropeptide modulation of vocal signaling

Reproduction in frogs is seasonal and, as in most vertebrates, modulated by circulating hormones and central neuropeptides (reviewed by Yamaguchi and Kelley [29]). We would thus expect that neural and muscular effectors for courtship songs would be sensitive to, for example, gonadal hormones and this has proved to be the case [30]. Somewhat less obvious is the part that acoustic stimulation plays for the endocrine system. For example, when male Southern leopard frogs were exposed to conspecific advertisement calls for 11 consecutive evenings, the conspecific call-stimulated group had higher levels of circulating gonadal androgens when compared with a control condition (a spectrally altered chorus broadcast) [31]. This effect of acoustic stimulation could be modulated by acoustic input to forebrain (the ventral striatum via the thalamic nuclei, discussed above). The ventral striatum has connections with preoptic area and infundibulum, regions that control pituitary function in other vertebrates [26,32].

We must now add social cues to the list of modulators of reproductive activity. The neuropeptide arginine vasotocin (AVT) increases pulse duration and number of pulses in a grey treefrog call when administered systemically [33]. The effect of AVT on males vocalizing in the field only occurs when males are close to (50 cm from) another calling male; AVT is ineffective on distant (2 meters away) males [34•].

Given the current lack of understanding of how vocal patterns are generated by the CNS, it is perhaps not surprising that we do not understand how androgens or AVT exert their effects on vocal behavior. However, it is assumed that at least part of the action occurs via the CNS where AVT-positive neurons and androgen receptors are found [35,36]. These hormonal effects are probably not independent; androgen modulates AVT expression in bullfrog CNS [37].

Some insight into neuro–hormonal mechanisms has been achieved through examination of the vocal neuromuscular synapse in *X. laevis*. Synapses of laryngeal motor neurons on laryngeal muscle fibers are weak in males and strong in females; the weak male synapses facilitate as trills are produced and contribute to the intensity modulation of the male's advertisement call [38], which is an attractive feature to females. Strong female synapses are the result of circulating estrogen [39,40]. Because estrogen effects are presynaptic but vocal motor neurons do not express estrogen receptors (ERs), the site of action of the hormone has been unclear. Using reverse transcriptase–polymerase chain reaction, northern and western blots and immunocytochemistry, the presence of ER protein and mRNA in laryngeal muscle fibers was confirmed [41•]. ER expression might mediate estrogen-dependent changes in synaptic strength via retrograde signaling. The primary ER expressed in *X. laevis* laryngeal muscle is the novel gene *xlERα2*; ERα1 is primarily expressed in liver, forebrain, and oviduct [42•]. *xlERα1* and *xlERα2* represent the first discovery of retained duplicates of the ERα gene in any species. Complementary spatial expression of these two genes and their alternatively spliced transcripts might have contributed to their conservation over such a long period of time, consistent with the subfunctionalization model for evolution after gene duplication.

Summary and conclusions

Why frogs? Given the strong conservation of molecular mechanisms from fly to man, and the conservatism of the nervous system with respect to sensory coding and neuroendocrine mechanisms, it should come as no surprise to discover mechanisms shared among slimy, feathered and furred species. A good example is the temporal coding of sounds, an essential feature both of human speech and of frog song. We are beginning to understand temporal production of song in *X. laevis* and decoding is being unraveled in Pacific tree frogs. Is frog song special or can we generalize? We know that brainstem mechanisms are highly conserved across vertebrates as are the general principles of vocal production so there is reason for optimism. What about binding? The existence of the McGurk effect in our own species has provided some support for, among other hypotheses, the gestural theory of the origin of language. As is the case for a great many attempts to tie human speech to species-specific perceptual features (e.g. categorical perception), auditory–visual binding might turn out to be a broad feature of vertebrate perception rather than a specifically human attribute. Finally, what about hormonal mechanisms and sex differences? The neuroendocrine basis of social recognition is currently a hot topic in mammalian cognitive neuroscience (please also see the article in this volume by Keverne and Curley; [43•]) and, as in frog vocal signaling, has been shown to involve the neuropeptides oxytocin and vasopressin; these are modulated by gonadal steroids. Do we, similar to voles, develop enduring attachments because of activation of central neuropeptides? Possibly, and, if so, modulation of neuropeptides and frog courtship song could have larger implications.

Acknowledgements

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- •• of outstanding interest

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The function of the vocal sac has been somewhat enigmatic from the acoustical point of view. Here, the authors ask whether or not an inflated sac contributes to call attractiveness. Male calls (whine plus the attraction-added chuck) were broadcast from the same spatial location as one of three possible visual stimuli: a male with an inflated sac, a male with an uninflated sac or an animated rectangle mimicking the horizontal movement of the sac. Only the inflated sac increased the attractiveness of the call. The authors argue that this visual stimulus increases a female's ability to localize a particular calling male in a dense chorus.

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In a study combining biorobotics and field biology, the authors induced male frogs to attack a robot (the electromechanical frog) but only when the robot is calling and if the sac is mechanically inflated. In a follow up study [10], they explore how close together sound and sight have to be to elicit attack and compare their results to 'binding' (e.g. the famous McGurk effect [9]).

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In laboratory studies, the amount of advertising produced by one male was profoundly affected by the presence of another male. Pairing two sexually active males resulted in suppression of advertisement calling in one; suppression was released when males were isolated after pairing. Vocal dominance was achieved even in the absence of physical contact (clasping). The authors suggest that *X. laevis* males gain a reproductive advantage by competing for advertisement privileges and by vocally suppressing neighboring males.

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18. •• Edwards CJ, Rose GJ: **Interval-integration underlies amplitude modulation band-suppression selectivity in the anuran midbrain**. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 2003, **189**:907-914.

This is the most recent in a series of important studies on the neural mechanisms that underlie decoding of the timing of acoustic signals. The authors explored the idea that, similar to band-pass neurons, band-suppression neurons derive their temporal selectivity from interval integration (i.e. counting). They do. Band-suppression neurons respond to both low and high PRRs because they have low thresholds for spike number and relatively large interval tolerances; at high PRRs discharges are phased-locked to the pulses. The authors suggest a mechanism for counting in an earlier study [20].

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In frogs, spatial unmasking is more robust at the torus semicircularis than at the auditory nerve. The authors studied toral neurons before and during iontophoretic application of bicuculline, a GABA receptor antagonist. Bicuculline reduced the strength of spatial unmasking of toral neurons to that characteristic of the auditory periphery. The results suggest that central inhibitory processes underlie the ability of the CNS to separate sound sources (unmasking).

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The authors compared the effects of lesions in the dorsal thalamus to those in the torus in a phonotaxis behavioral paradigm using female treefrogs. Toral lesions abolished or reversed call preference, whereas dorsomedial thalamic lesions were without effect. Thalamic nuclei might have motivational or attentional functions.

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27. Yamaguchi A, Kelley DB: **Generating sexually differentiated vocal patterns: laryngeal nerve and EMG recordings from vocalizing male and female African clawed frogs (*Xenopus laevis*)**. *J Neurosci* 2000, **20**:1559-1567.

28. •• Yamaguchi A, Kaczmarek L, Kelley DB: **Functional specialization of male and female motoneurons**. *J Neurosci* 2003, **23**:11568-11576.

The authors use a 'vocal slice' preparation to explore sexually differentiated properties of laryngeal motor neurons with voltage and current clamps. Male motor neurons have lower input resistances and larger membrane capacitances than female motor neurons. The strongly adapting neurons that predominate in males initiate spikes at short reliable latencies, whereas the weakly adapting motor neurons characteristic of females translate graded levels of depolarization into different firing rates. Low-threshold potassium currents predominate in males. Hyperpolarization-activated cationic currents are found almost exclusively in males. Modeling results indicate that sex-typical active and passive properties can account for the occurrence of strongly and weakly adapting spike trains in the sexes. Physiological properties are matched to sex-typical vocal demands.

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AVT affects the production of advertisement calls and the outcome of competitive interactions among male gray treefrogs. The authors demonstrate that AVT does not influence call duration and pulse number unless another male is nearby. The results add to a growing body of evidence implicating AVT in social cognition [43•].

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The authors identify two distinct ERα genes, *xlERα1* and *xlERα2*, the first example of retained duplicates of the ERα gene in any species. The two xlERα loci were generated by a duplication specific to the *X. laevis* lineage — probably the genome duplication that led to a doubling of the *X. laevis* chromosome number about 30 million years ago. The primary ER expressed in *X. laevis* laryngeal muscle is the novel gene *xlERα2* [42•].

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The polymerase chain reaction and northern blots revealed ER transcipts in laryngeal muscle. Immunocytochemistry and western blots confirmed the presence of ER protein. The ER could mediate changes in synaptic strength via retrograde signaling.

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This is a review of recent literature on the role of neuropeptides and gonadal hormones in the recognition process that underlies social familiarity. The authors bring together extensive literature implicating neuropeptides in learning and memory with evidence — from Insel and Young's work — that neuropeptide secretion and receptor distribution is implicated in pair bonding. The implication is that neuropeptide signaling, and its modulation by gonadal steroids, subserves a highly selected process for the recognition of salient conspecifics. This system might be an elaboration of a similar one in frogs that impacts male–male aggressive interactions (see Trainor *et al*. [34•]).