

peptide ( $\Delta$ SP-CKX2) and colocalizes with a cytoplasmic marker (fig. S7D) did not affect the cytokinin response, and neither did CKX7, which also localizes to the cytoplasm (25) (fig. S7E). Crude cell extracts obtained from  $\Delta$ SP-CKX2-transfected or CKX7-transfected cells added to the medium reduced the response triggered by tZ, indicating that these proteins are active. These data suggest that apoplastic cytokinins initiate signaling, whereas cytoplasmic cytokinins are inactive.

PUP14 imports cytokinins from the apoplast to the cytosol, away from sensing domains of plasma membrane-localized receptors, which causes a reduction in cytokinin signaling. Thus, PUP14 activity inversely correlates with the capacity of a cell to sense cytokinins (fig. S8), and PUP14 spatio-temporal activities cause region-specific depletion of cytokinins from the apoplast. In animals, the importance of such clearing activities is demonstrated by the powerful action of drugs that target dopamine influx transporters, thereby increasing dopamine's residence time in the synaptic cleft (26). Feeding experiments with radiolabeled bioactive cytokinin suggest that the bulk of imported cytokinins are inactivated by conversion to monophosphates by ADENINE PHOSPHORIBOSYL TRANSFERASE enzymes (27, 28). Furthermore, N- or O-glycosylation, oxidative cleavage, or transport to other cells may contribute to clearance of intracellular cytokinins (2). PUP14 is the only family member to be linked to cytokinin signaling in all organs. In specific developmental contexts, additional PUP family members likely have overlapping functions with PUP14. The fact that PUP genes are specific to vascular plants (29) may suggest that PUP genes are needed to support more complex cytokinin signaling patterns associated with the bauplan of land plants. Because hormonal transporters are numerous and universal in plant and animal systems, transporters in other systems may also regulate patterning during morphogenesis.

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## SUPPLEMENTARY MATERIALS

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## SPEECH PROCESSING

# Neural mechanisms for lexical processing in dogs

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**During speech processing, human listeners can separately analyze lexical and intonational cues to arrive at a unified representation of communicative content. The evolution of this capacity can be best investigated by comparative studies. Using functional magnetic resonance imaging, we explored whether and how dog brains segregate and integrate lexical and intonational information. We found a hemispheric bias for processing meaningful words, independently of intonation; an auditory brain region for distinguishing intonationally marked and unmarked words; and increased activity in primary reward regions only when both lexical and intonational information were consistent with praise. Neural mechanisms to separately analyze and integrate word meaning and intonation in dogs suggest that this capacity can evolve in the absence of language.**

**V**arious species rely on similar acoustic cues from vocalizations to infer inner states (1, 2). Human vocal comprehension also uses association of arbitrary sound sequences with meaning. Lexical items (words) are the basic building blocks of human languages but are hardly ever found in nonhuman vocal communicative systems, even though several species are capable of learning and discriminating arbitrary sound sequences (3, 4), associating vocalizations with specific meanings (4, 5), or producing human-like lexical items after extensive training (6).

Lexical processing in humans is lateralized to the left hemisphere (LH) of the brain (7). According to acoustic theories, this is caused by LH bias for rapidly changing signals (8), which is not unique to humans (9). In contrast, functional theories assume hemispheric bias for lexical representations of meaning, independent of acoustics (10). Nonhuman neural evidence for lexical processing is scarce. Hemispheric bias for

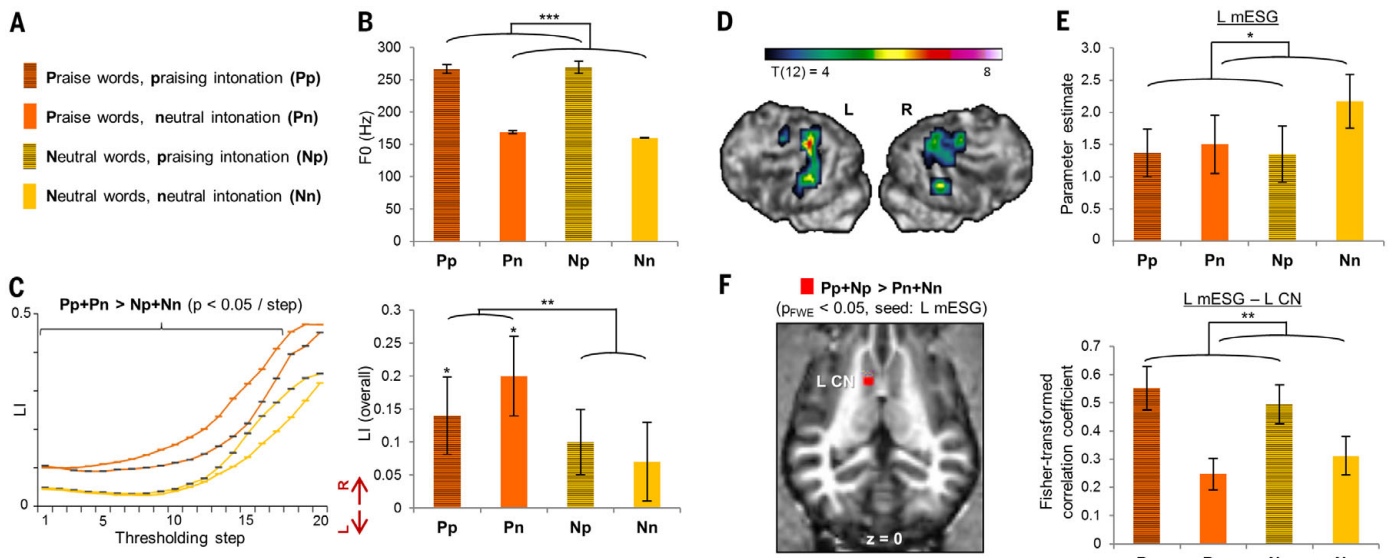
broadly defined meaningfulness has been found for processing familiar, conspecific sounds (11–13). The comparison of human and nonhuman neural mechanisms for processing spoken words may reveal how speech-related hemispheric asymmetries and lexical representations emerged during evolution.

Dogs present an ideal model for such investigations. Domestication has increased dogs' abilities to engage in acoustic communication with humans (14), they are more receptive than wolves to human vocal signals (15), and their behavior is more easily brought under human vocal control (16, 17). Dogs can recognize (up to ~1000) words as discriminative stimuli to retrieve different objects (18), and they process acoustic cues from human and dog vocalizations in overlapping auditory brain regions (19). A recent behavioral study found orienting asymmetries in dogs that listened to artificially manipulated spoken commands, providing indirect evidence of hemispheric bias when the salience of meaningful phonemic cues increased (20).

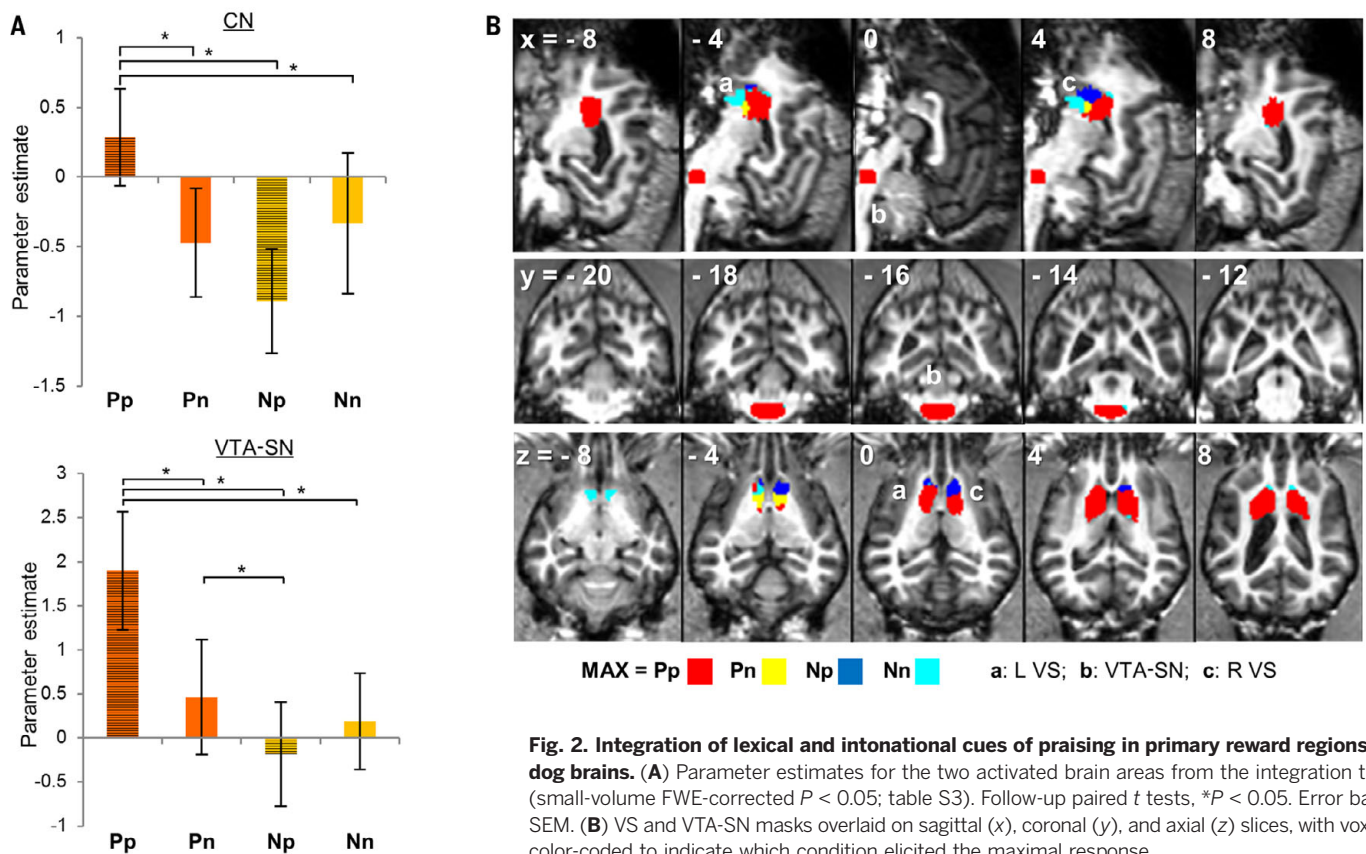
We applied functional magnetic resonance imaging to disentangle lexical versus intonational processing in awake dogs (19) (fig. S1). Verbal praises were used as stimuli because (i) human languages signal praise both lexically (“Well done!”) and intonationally [higher pitch and pitch range, specific pitch contour (21)]; (ii)

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**Fig. 1. Distinct neural patterns for lexical and intonation processing in dog brains.** (A) Experimental conditions. (B) Acoustic variation of stimuli. (C) Hemispheric lateralization test. Lateralization indices (LI) are shown across thresholds (connecting lines) and overall (horizontal bars). Positive values, right hemisphere. (D) Dog auditory regions responsive to speech (table S2). The color bar shows the range for one-sample *t*-test scores (12 degrees of freedom) for the speech (Pp + Pn + Np + Nn) > silence contrast. (E) Random-effects tests in auditory regions. (F) Functional connectivity tests. Random-effects test results are superimposed on an axial (*z*) slice [whole-brain familywise error (FWE)-corrected  $P < 0.05$ ]; table S4].  $N = 13$ ;  $***P < 0.001$ ;  $**P < 0.01$ ;  $*P < 0.05$ ; error bars, SEM.



**Fig. 2. Integration of lexical and intonational cues of praising in primary reward regions of dog brains.** (A) Parameter estimates for the two activated brain areas from the integration test (small-volume FWE-corrected  $P < 0.05$ ; table S3). Follow-up paired *t* tests,  $*P < 0.05$ . Error bars, SEM. (B) VS and VTA-SN masks overlaid on sagittal (*x*), coronal (*y*), and axial (*z*) slices, with voxels color-coded to indicate which condition elicited the maximal response.

verbal praises are often used in dog-directed speech as social rewards; and (iii) neural evidence on reward processing mechanisms is well established (22, 23). Primary reward regions (the mesolimbic dopamine system), consisting of the

ventral striatum (VS) and dopamine neurons of the ventral tegmental area and substantia nigra (VTA-SN) (24, 25), consistently respond more strongly to reward than to nonreward signals in humans, and this is also the case in dogs (26).

We manipulated lexical information (marked, praise word; unmarked, neutral word) and intonation (marked, praising tone; unmarked, neutral tone) separately. Experimental conditions involved every combination of word type and

intonation (Fig. 1A): praise words in praising intonation (Pp), praise words in neutral intonation (Pn), neutral words in praising intonation (Np), and neutral words in neutral intonation (Nn). For praise words, we selected Hungarian expressions used by all test dog owners for praising. For neutral words, we used Hungarian conjunction words of similar frequency. Typically, praise but not neutral words are spoken with praising intonation, and only Pp is used to address dogs. We assumed that Pp is meaningful to dogs whereas Nn is not, and that praise words thus contain lexical cues but neutral words do not.

We hypothesized that if dogs maintain lexical representations, neural reward responses would depend on both lexical and intonational information. In contrast, if dogs do not segregate lexical information from intonation, neural reward responses would be modulated only by intonational cues. Hemispheric bias for lexical processing would be consistent with functional theories, no lateralization for lexical cues and RH bias for intonational cues would support the acoustic account, and hemispheric bias for Pp (and perhaps Nn) relative to Pn and Np would argue for a role of familiarity.

Vocal stimuli from the dogs' female trainer were recorded. We used this single individual's voice, which was similarly well known to every dog, because speaker familiarity affects dogs' reactions to verbal utterances (27). Stimuli with praising intonation had a higher and more varying pitch, but no systematic acoustic differences were found between praise and neutral words (Fig. 1B). Non-Hungarian listeners scored intonationally but not lexically marked stimuli as more praising (28).

To assess overall lateralization, we used a bootstrapping approach with multiple cutoff thresholds (29) (Fig. 1C and table S1). Cortical responses for lexically marked (Pp and Pn) but not unmarked (Np and Nn) words were RH-lateralized. Hemispheric bias for lexically marked words was persistent across thresholds. There were no intonational or lexical-intonational effects. In a previous study (19), we found no lateralization bias for non-speech human sounds. These findings support the functional account (10, 20) and suggest that dog brains maintain intonation-independent lexical representations of meaning. Lateralization for Pp could also be related to its higher frequency in dog-directed speech, but Pn, though rare, elicits a similar hemispheric bias, making a familiarity-based account improbable.

To investigate regional effects, we performed random-effects tests, focusing on two sets of brain areas: (i) auditory regions responsive to speech (Fig. 1D and table S2), localized functionally, and (ii) primary reward regions, VS and VTA-SN (24, 25), defined anatomically (30). We tested for separate effects of lexical (Pp + Pn versus Np + Nn) and intonational (Pp + Np versus Pn + Nn) processing and for combined effects of lexical-intonational processing in three ways: an interaction test [(Pp versus Pn) versus (Np versus Nn)], a simple contrast or "integration" test (Pp versus Pn + Np + Nn), and a conjunction test [(Pp versus Pn)  $\cap$  (Pp versus Np)].

Within auditory regions, we found intonation effects but no lexical or lexical-intonational effects.

Intonation effects were only evident in the left middle ectosylvian gyrus (L mESG), with stronger responses for words with neutral intonation, independent of word meaning (Fig. 1E and table S3). We then modeled acoustic variation across stimuli, using parametric modulators. We found that L mESG activity negatively covaried with fundamental frequency (F0), paralleling findings of higher sensitivity to lower pitch in the near-primary auditory cortex in humans (31), macaques (32), and dogs (19). There was no intonation or F0 effect in the right homolog region (R mESG) but also no significant hemispheric bias (2, 28). Unlike in human studies, we found no effect of F0 change (33) (table S3). In whole-brain condition-dependent functional connectivity analyses with auditory subregions as seeds, an intonation effect (stronger correlation for praising intonation) was only found between the L mESG (as the seed) and L caudate nucleus (CN; Fig. 1F and table S4). Connectivity results suggest a human-analog functional link between auditory and reward regions for processing praising intonation (34). The L mESG in dogs is thus involved in processing acoustic cues that are relevant for emotional intonation in both speech and nonspeech human and dog vocalizations (19). Analogously, emotional intonation processing in humans involves mechanisms that are not specific to speech (35–37).

In primary reward regions, we found combined lexical-intonational effects but no separate lexical or intonational effects. Pp elicited stronger neural responses than any other condition in the dopamine nuclei of the VTA-SN and in the CN (within the VS) (Fig. 2A and table S3). Dog reward regions thus respond most strongly to verbal praises when both lexical and intonational information fit. To further illustrate these findings, we calculated the maximal response for each voxel within the reward masks: Pp had the highest percentages in both the VS (Pp, Pn, Np, and Nn: 71.3, 6.2, 8.6, and 13.9%) and the VTA-SN (96.0, 0.0, 0.0, and 4.0%) (Fig. 2B). Similarly to humans, dogs appear to integrate lexical and intonational cues in speech to evaluate meaning in nonauditory brain regions (38).

We discovered three neural mechanisms of speech processing in dogs. First, there was a hemispheric bias for processing meaningful words, independently of intonation. Second, acoustic cues of affective speech intonation were processed independently of word meaning in mESG, and intonational markedness increased functional connectivity between auditory and caudate regions. Third, dogs relied on both word meaning and intonation when processing the reward value of verbal utterances. All three findings reveal functional analogies between dog and human brain mechanisms (2, 7, 19, 34, 38). We suggest that in a suitable ontogenetic environment, lexical representations can arise and be separated from acoustics, even in a nonprimate mammal. In dogs, specific selective forces during domestication could have also supported interspecific communicative and learning skills (17), but rapid evolution of speech-related hemispheric asymmetries is unlikely (39). Lateralized lexical processing does

not appear to be a uniquely human capacity that follows from the emergence of language, but rather a more ancient function that can be exploited to link arbitrary sound sequences to meanings. What makes lexical items uniquely human is thus not the neural capacity to process them, but the invention of using them.

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