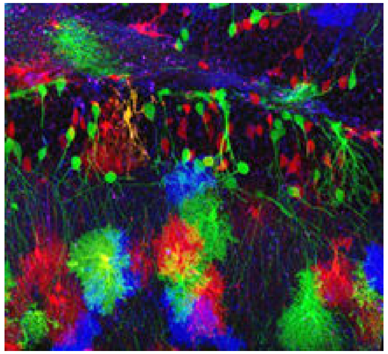
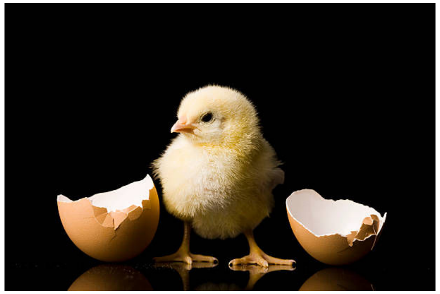
大国主命と縁結



ブレンボー脳 虹

言葉（発声）の脳科学
（言葉・発声、遺伝子、脳・神経）

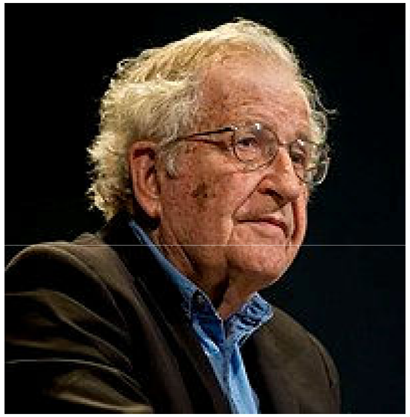


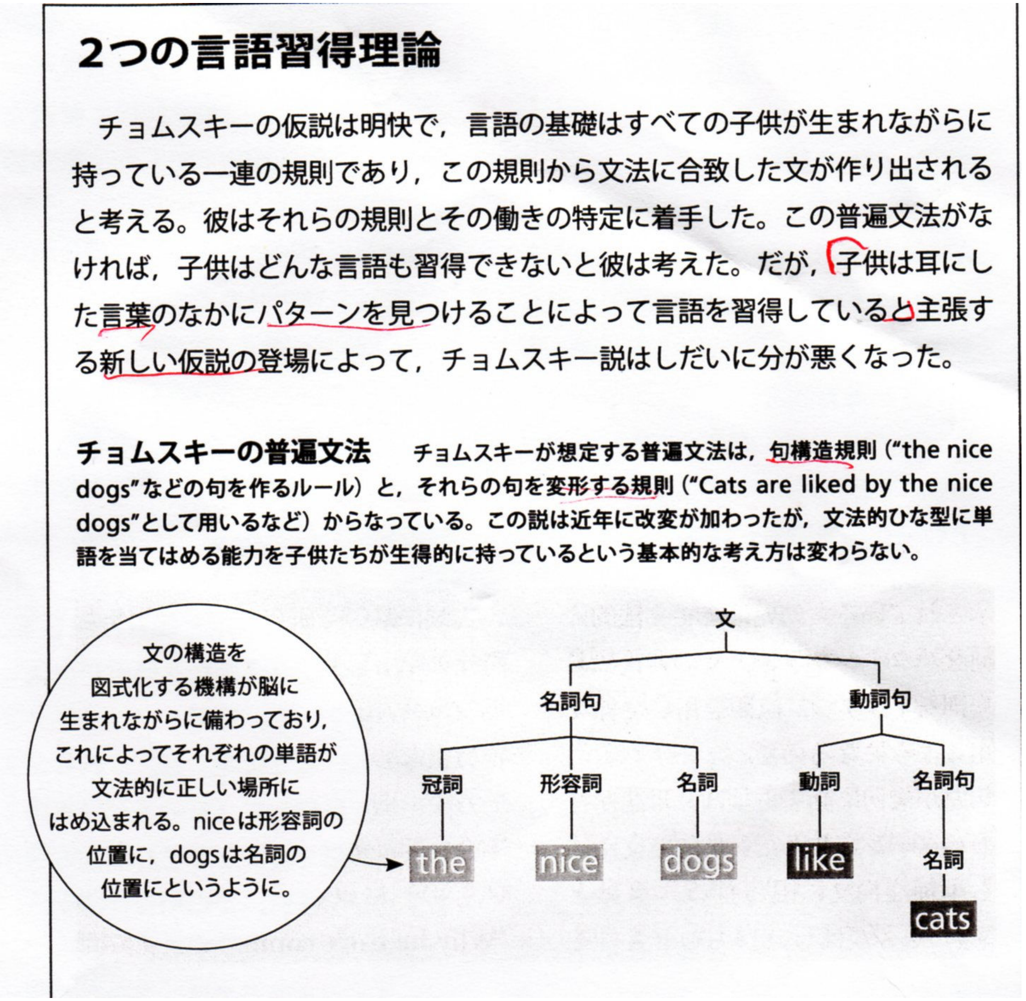
別所　親房

（天地　旅人)

私とヨーコ

1.言語習得理論





2つの言語習得理論

チョムスキーの仮説は明快で，言語の基礎はすべての子供が生まれながらに持っている一連の規則であり，この規則から文法に合致した文が作り出されると考える。彼はそれらの規則とその働きの特定に着手した。この普遍文法がなければ，子供はどんな言語も習得できないと彼は考えた。だが，子供は耳にした言葉のなかにパターンを見つけることによって言語を習得していると主張する新しい仮説の登場によって，チョムスキー説はしだいに分が悪くなった。

チョムスキーの普遍文法　チョムスキーが想定する普遍文法は，句構造規則（“the nice dogs”などの句を作るルール）と，それらの句を変形する規則（“Cats are liked by the nice dogs”として用いるなど）からなっている。この説は近年に改変が加わったが，文法的ひな型に単語を当てはめる能力を子供たちが生得的に持っているという基本的な考え方は変わらない。

文の構造を図式化する機構が脳に生まれながらに備わっており，これによってそれぞれの単語が文法的に正しい場所にはめ込まれる。niceは形容詞の位置に，dogsは名詞の位置にというように。

2. 言語（障害）と*FOXP2*遺伝子

C.S. Lai et al.(2001) Nature 413 519-523

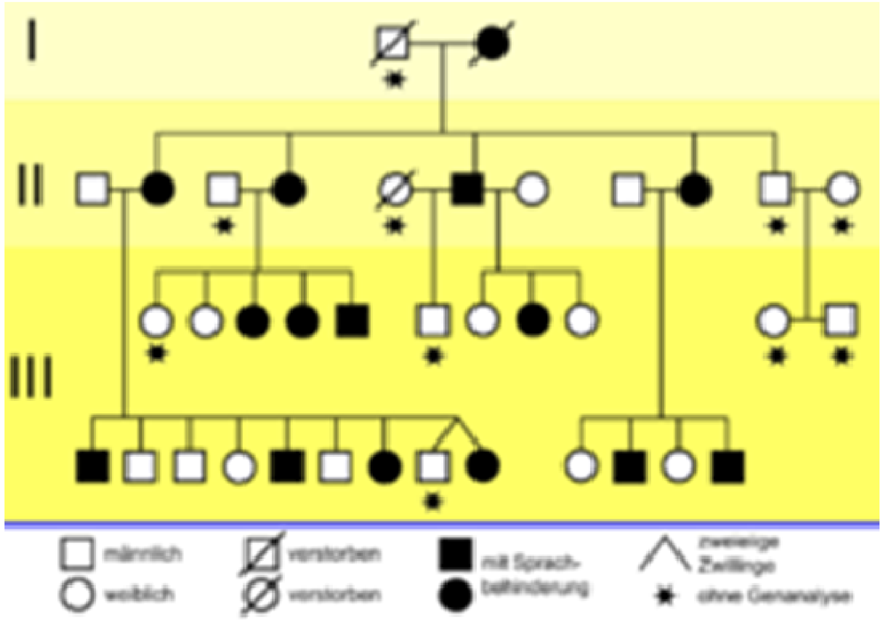


図2 KE家の言語障害: 活用語尾の
使用障害、知能指数や口の運動障害、脳の
発達異常（線条体、ブローカ野が小さい）

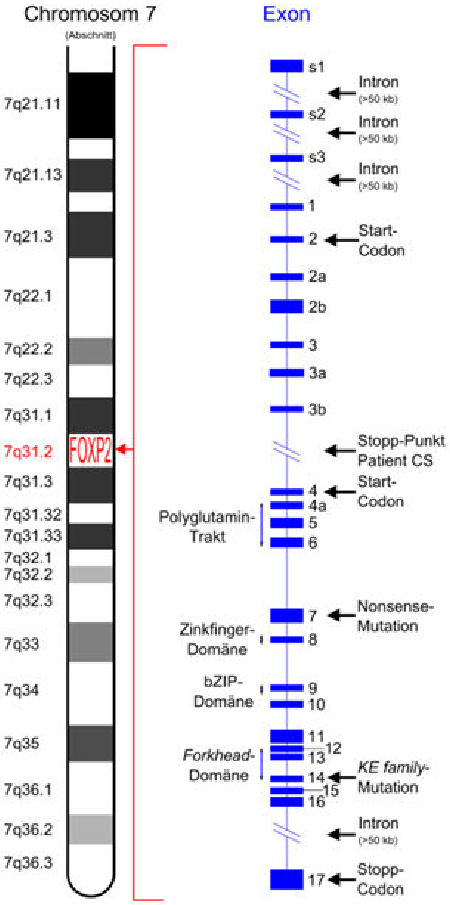
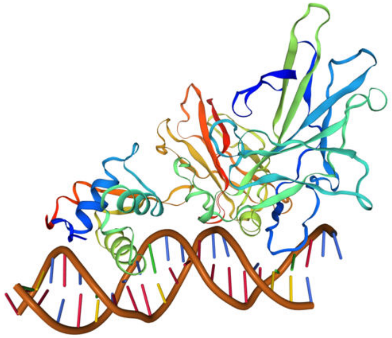


図3 K E家の染色体・遺伝子解析

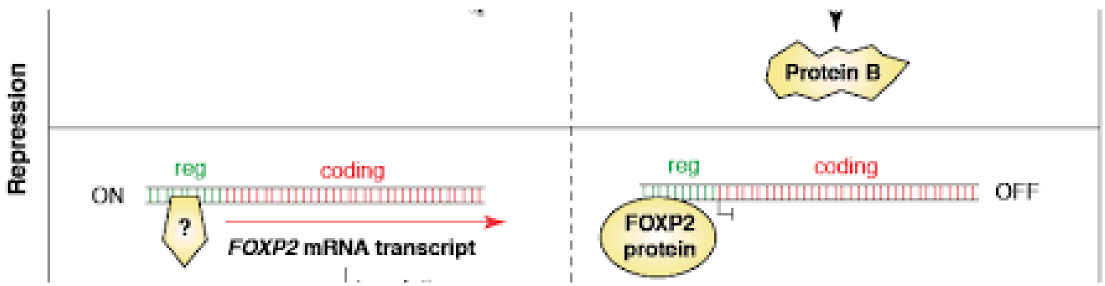
Cecilia et al. Nature 413, 519-523 (2001)

3. FOX（P2）とは？

- **FOX**(Forkhead box)蛋白; 転写因子ファミリー、ホメオ遺伝子（フォーク頭）は核タンパク質をコードし、ショウジョウバエの胚末端に発現する。Wegel et al. (1989)　神経伸長
- FOX遺伝子;　homeo geneの一種、A-R 18種、foxp1 (多能性、脳と肺), **foxp2** (脳、言語), foxp3 (T細胞), foxp4

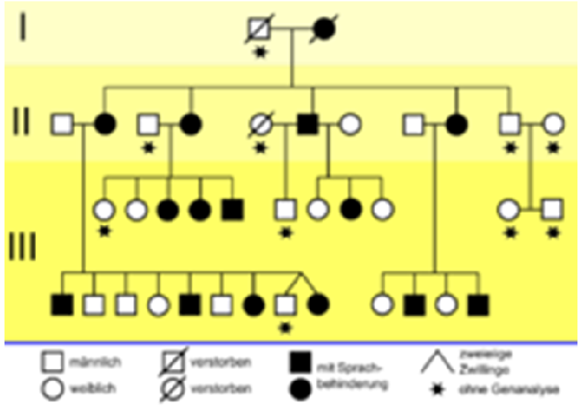


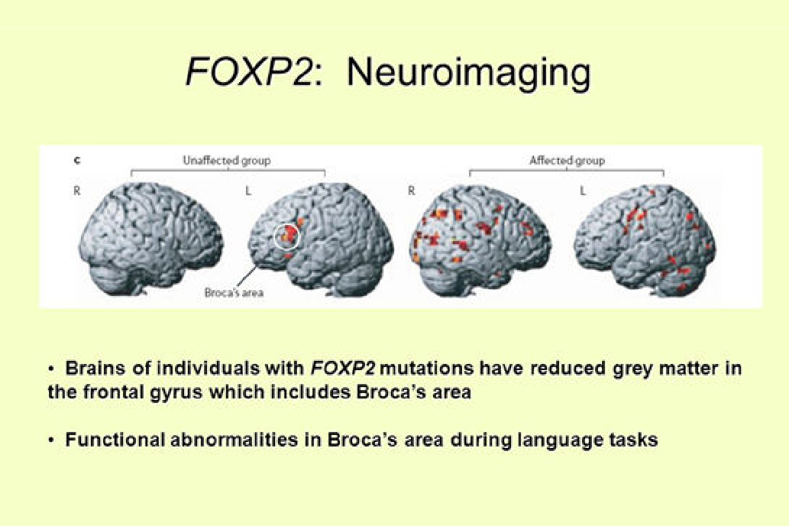
FoxP2のDNA転写開始部位結合モデル



FoxP2の転写抑制モデル

4. KE家の人脳画像とヒト脳でのFOXP2発現

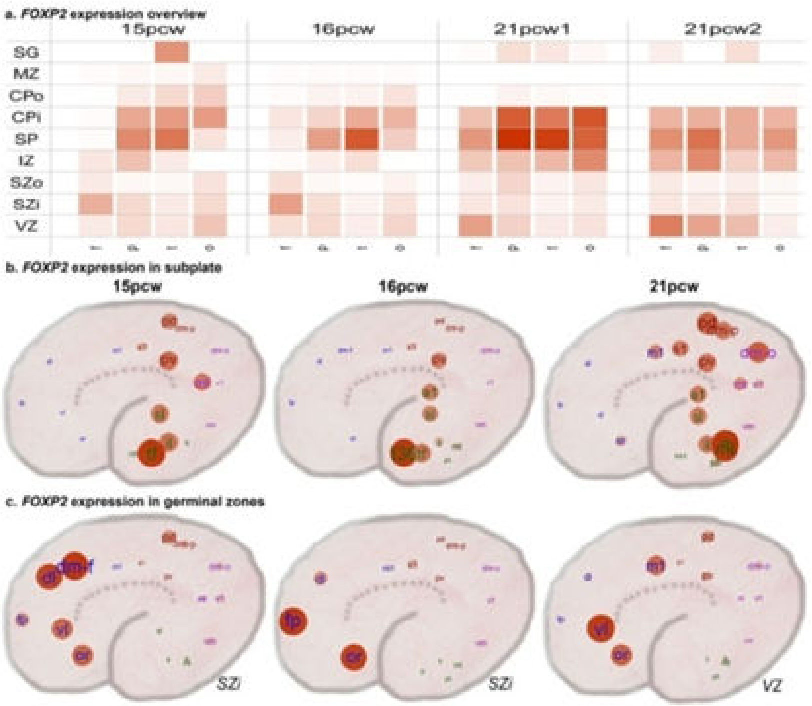




FOXP2: Neuroimaging

- Brains of individuals with *FOXP2* mutations have reduced grey matter in the frontal gyrus which includes Broca's area
- Functional abnormalities in Broca's area during language tasks

KE家の人の右左活性像 fMRI
(Nature Neuroscience (2003))



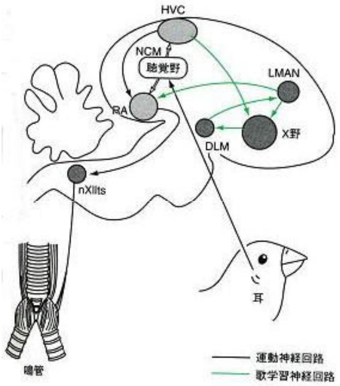
ヒト胎児脳での*FOXP2*発現分布 ISH
(Nature 508,199-206 (2014))

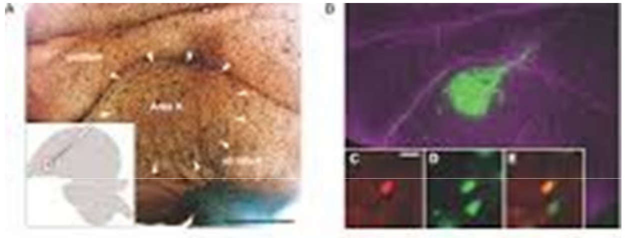
5.キンカチョウZFの領域XでのFoxP2ノックダウンKD（レンチ ウイルスを介したRNA干渉)は歌形式を変える

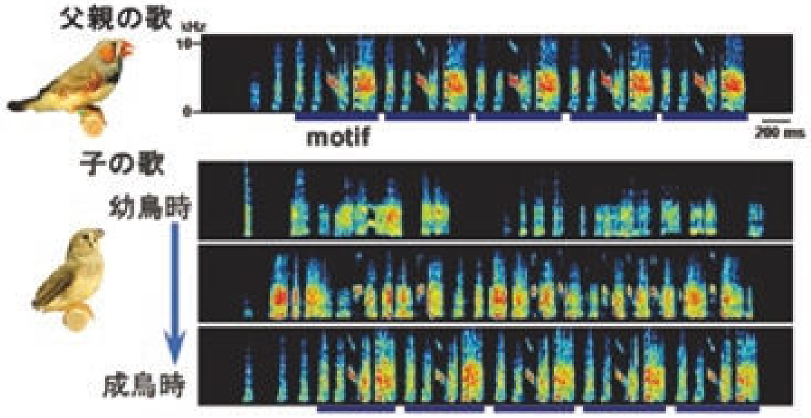
(S. Haesler et al. Incoplete and inacculate vocal imitation after knockdown of *FoxP2* in songbird basal ganglia nucleus Area X. PLoS Bio. 5 (2007) e321)

A. **発声の模倣学習VIL**: male zebra finch juvenile(pupil) and adult (tutor)

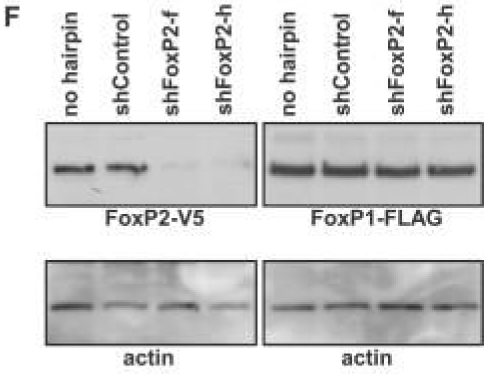
B. FoxP2 KDした鳥の不正確な歌模倣のソナグラムmale ZF with FoxP2 KD and adult ZF

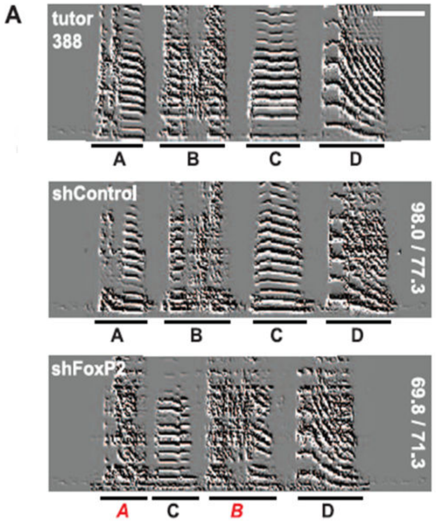






キンカチョウ雄の歌模倣学習発達の音声スペクトラム（和多和宏）





6. ヒト化マウス　ノックアウトとトランスジェニック法

W.Enard et al. S. Paabo (2009)A humanized version of Foxp2・・・,Cell 137,961-971

ミッキーマウス

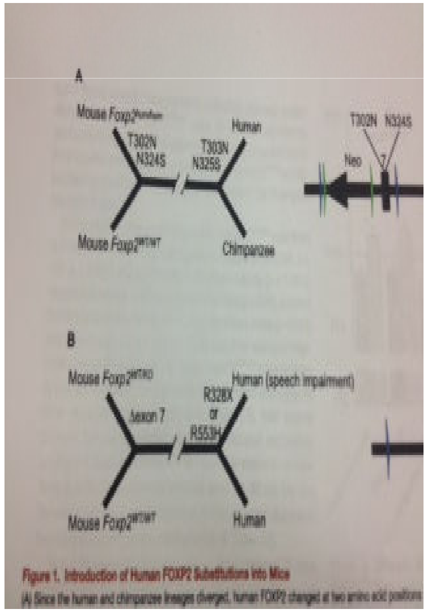
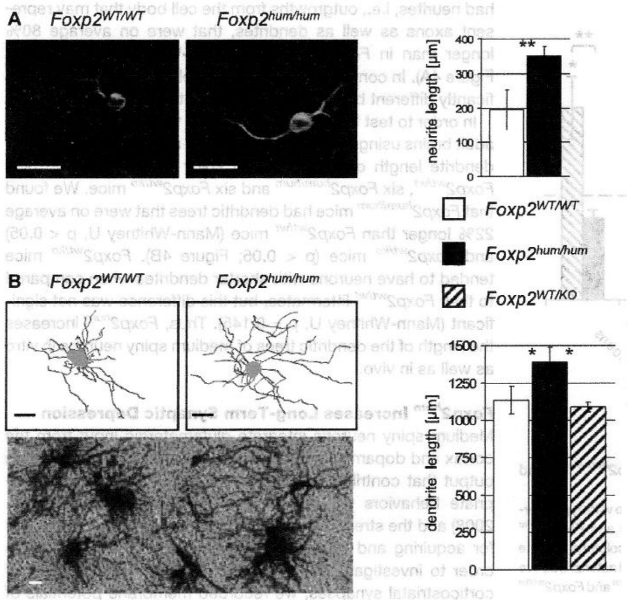


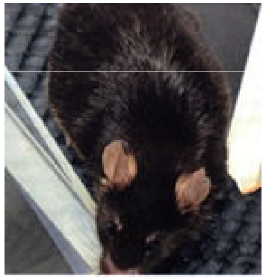
Figure 1. Introduction of Human FOXP2 Substitutions into Mice

(A) Since the human and chimpanzee lineages diverged, human FOXP2 changed at two amino acid positions

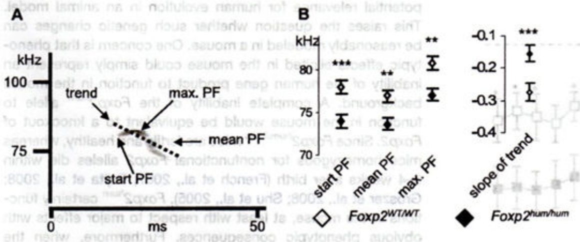
ヒト*FOXP2*導入マウス作製法



ヒト*FOXP2*導入マウスは樹上突起を伸ばす



FoxP2ヒト化マウス



ヒト化マウスの隔離コールは低音化

7. ニワトリとウズラのキメラ研究

E. Balaban et al.(1988) Science 241 1339-1342

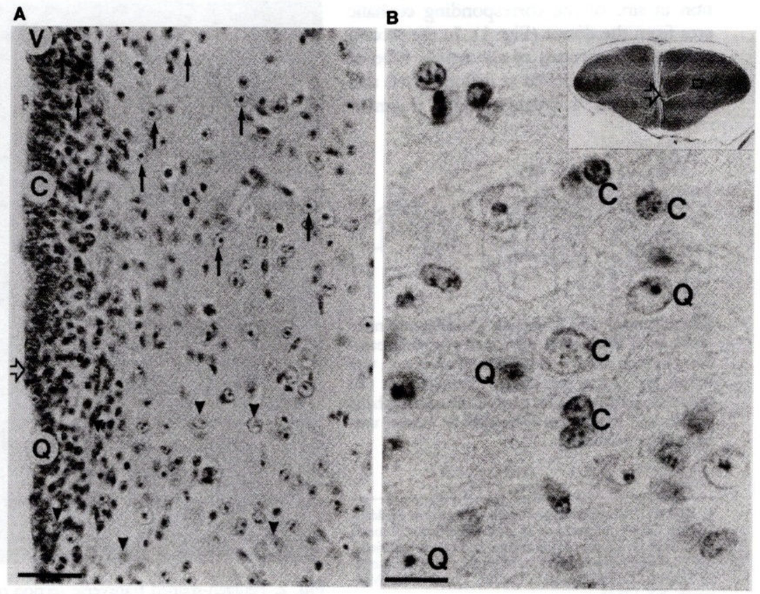


Fig. 4. Feulgen-stained section through the cerebral hemispheres of a quail (Q) into chick (C) chimera (experiment C in Fig. 1) at P7. (**A**) Host and donor cells are well separated at the level of the ventricular epithelium (V). A mixing of quail (↑) and chick (▼) cells immediately in contact with this thin cell layer indicates tangential cell movements during development; this produces truly chimeric brain regions. (**B**) Enlargement of the region indicated in the inset showing a mixed region containing quail and chick neurons and glial cells. Large arrowheads indicate the ventricular boundary of the graft. Bar in (A), 22 μm; bar in (B), 11 μm.

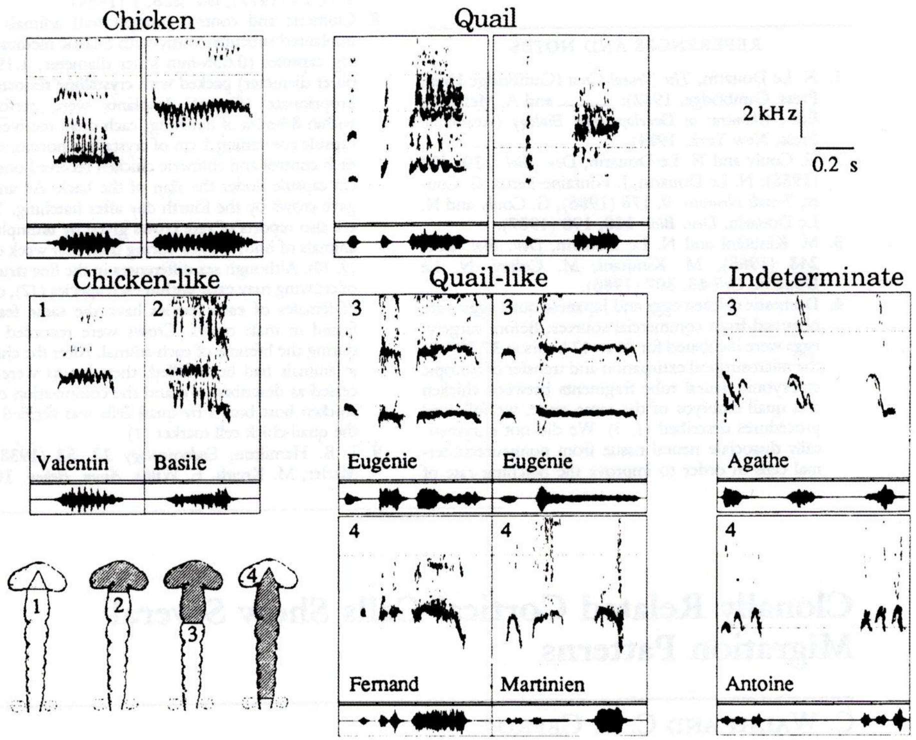


Fig. 5. Testosterone-induced juvenile crowing patterns from chickens, quails, and quail donor–chicken host brain chimeras. All crows shown were recorded between 4 and 7 days after hatching. Each box shows an amplitude-time (bottom) and frequency-time (sound spectrographic, top) representation of one crowing vocalization. Frequency and time markers are as indicated at the top right. Transplant operations are shown schematically at the lower left: 1, transplant of the dorsal neural tube primordium, giving rise to the dorsal thalamus; 2, transplant of the whole prosencephalic neural tube, giving rise to the entire telencephalon, diencephalon, and eyes; 3, transplant of the whole prosencephalic and mesencephalic neural tube, giving rise to the entire telencephalon, diencephalon, and mesencephalon including the eyes; or 4, transplant of the whole neural tube between the first somite and the caudal part of the prosencephalon, giving rise to the entire rhombencephalon, cerebellum, mesencephalon, diencephalon, and caudal portions of the telencephalon. Numbers in the upper left corner of each

ヒヨコとウズラ　ヒナの鳴き声

Valentin強い,Basileバジル,Eugenie人名,Agathe女性名,

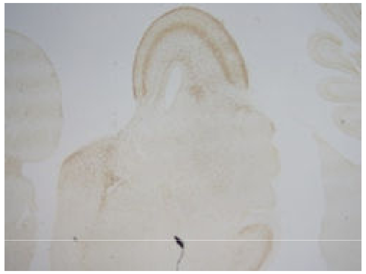
8. 遺伝子導入、Knock Downによるヒヨコの形質転換（神経回路と音声改変）

ヒト様に喋らせてみよう　トリ、マウス！

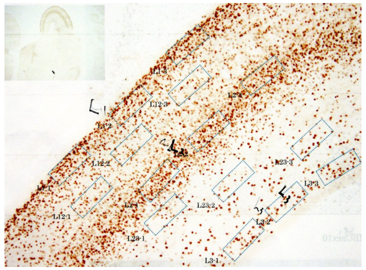
私の研究

FoxP2 decreases at a specific region of chick midbrain after hatchingの研究背景資料

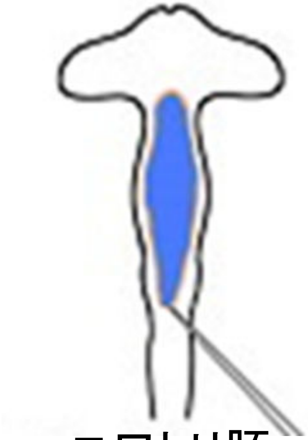
ドナルドダック



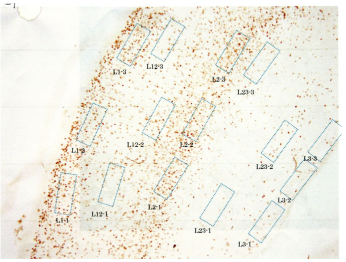
孵化前脳切片でのFoxP2発現分布



孵化前



ニワトリ胚への遺伝子導入

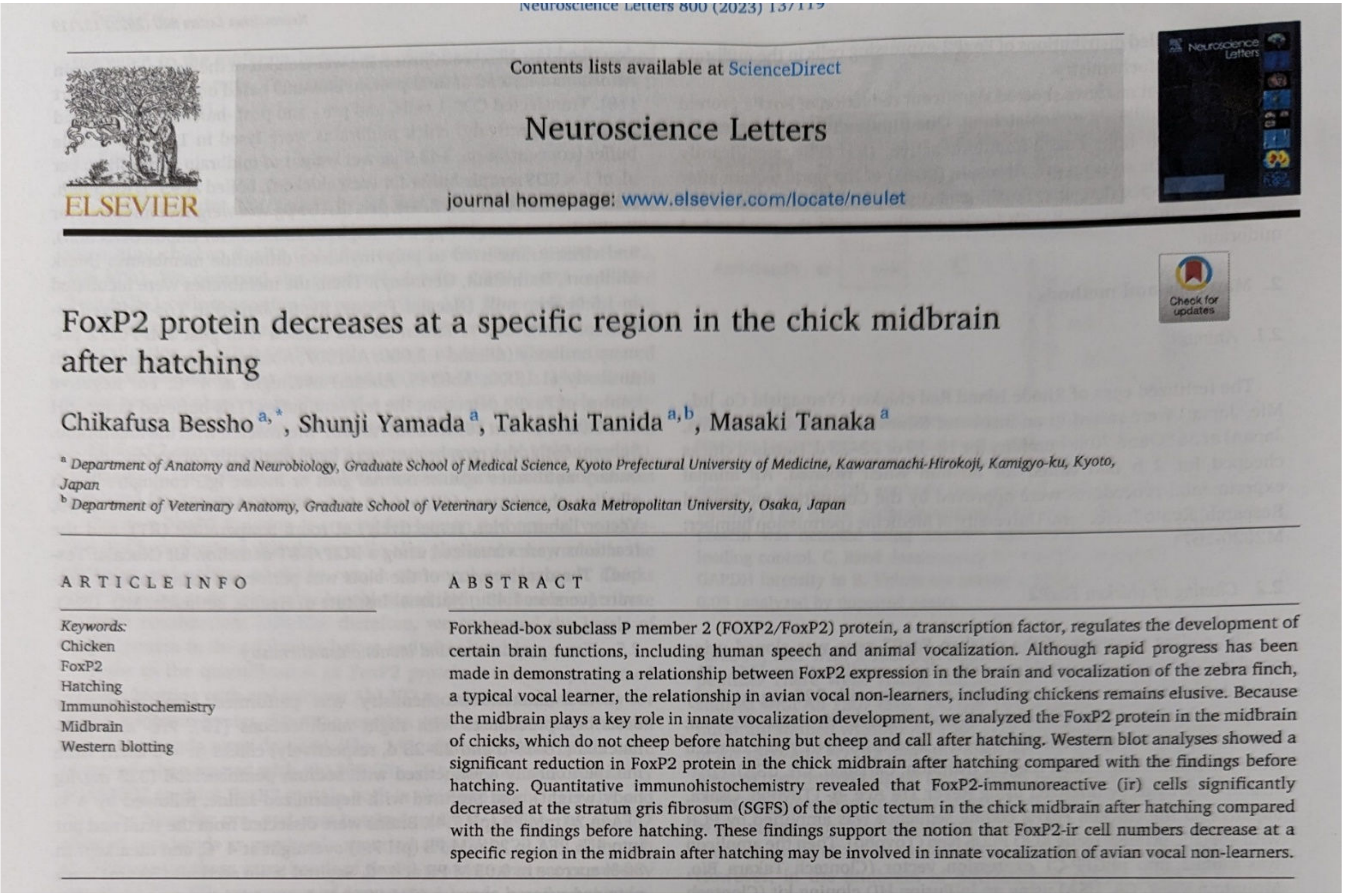


孵化後

ニワトリ中脳でのFoxP2たんぱく質の発現分布

9.FoxP2蛋白質はニワトリ卵孵化後　中脳の特別の領域で減少する

FoxP2 Protein decreases at a specific region in the chick midbrain after hatching



Neuroscience Letters 800 (2023) 137119

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FoxP2 protein decreases at a specific region in the chick midbrain after hatching

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ARTICLE INFO

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Hatching
Immunohistochemistry
Midbrain
Western blotting

ABSTRACT

Forkhead-box subclass P member 2 (FOXP2/FoxP2) protein, a transcription factor, regulates the development of certain brain functions, including human speech and animal vocalization. Although rapid progress has been made in demonstrating a relationship between FoxP2 expression in the brain and vocalization of the zebra finch, a typical vocal learner, the relationship in avian vocal non-learners, including chickens remains elusive. Because the midbrain plays a key role in innate vocalization development, we analyzed the FoxP2 protein in the midbrain of chicks, which do not cheep before hatching but cheep and call after hatching. Western blot analyses showed a significant reduction in FoxP2 protein in the chick midbrain after hatching compared with the findings before hatching. Quantitative immunohistochemistry revealed that FoxP2-immunoreactive (ir) cells significantly decreased at the stratum gris fibrosum (SGFS) of the optic tectum in the chick midbrain after hatching compared with the findings before hatching. These findings support the notion that FoxP2-ir cell numbers decrease at a specific region in the midbrain after hatching may be involved in innate vocalization of avian vocal non-learners.

10.ニワトリFoxp2蛋白質に市販の羊抗体FoxP2が使えるか？

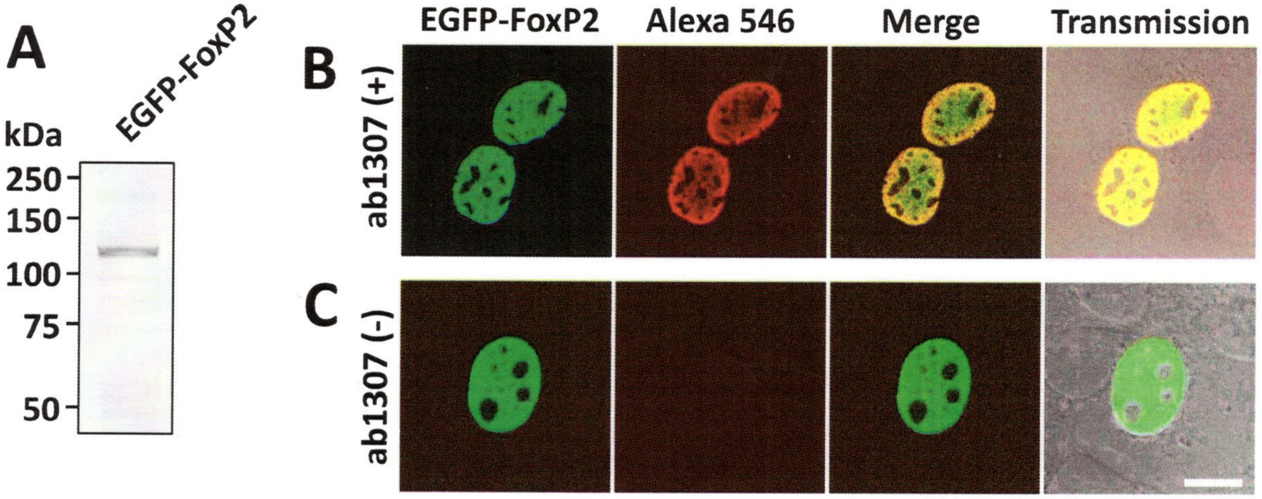
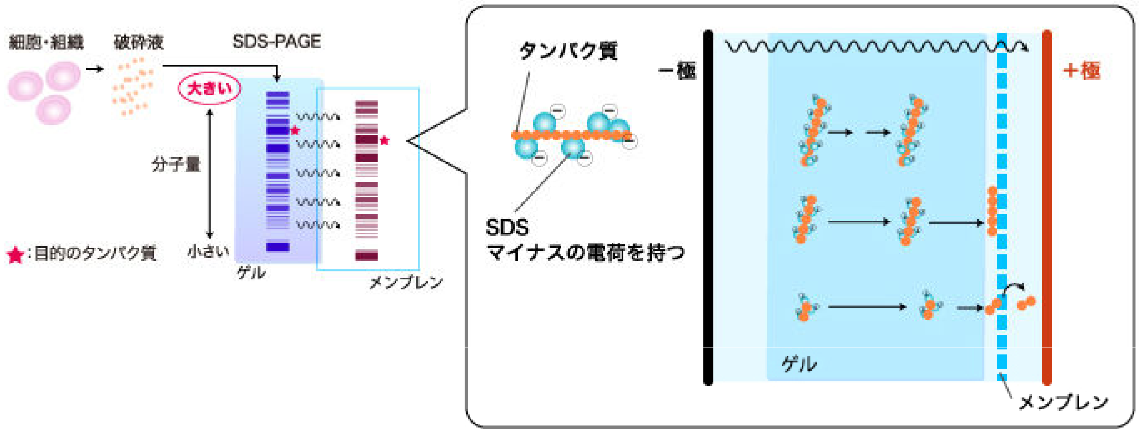
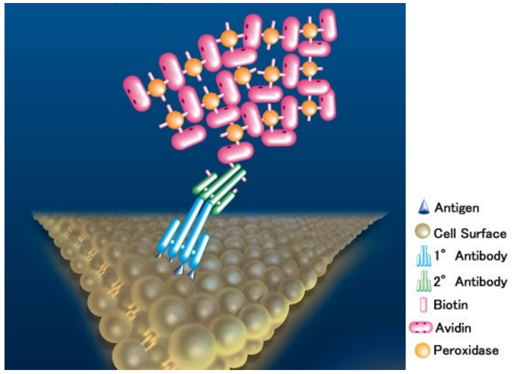


Fig. 1. Validation of anti-FoxP2 primary antibody Ab1307 for chick FoxP2 protein. A, Western blotting with Ab1307 on COS-1 cells expressing chick FoxP2 tagged with EGFP. B, C, Immunofluorescence cytochemistry. COS-1 cells expressing EGFP-tagged chick FoxP2 were fixed with 4 %PFA in 0.1 M PB, incubated with (B) or without (C) Ab1307, and reacted with Alexa Fluor 546-conjugated antibody. Images were acquired by confocal laser scanning microscopy. Scale bar = 20 μm.

11.ウエスタンブロッティングと免疫組織化学染色





12.ニワトリ有精卵のふ化前後で中脳のFoxP2蛋白質は変化するか？

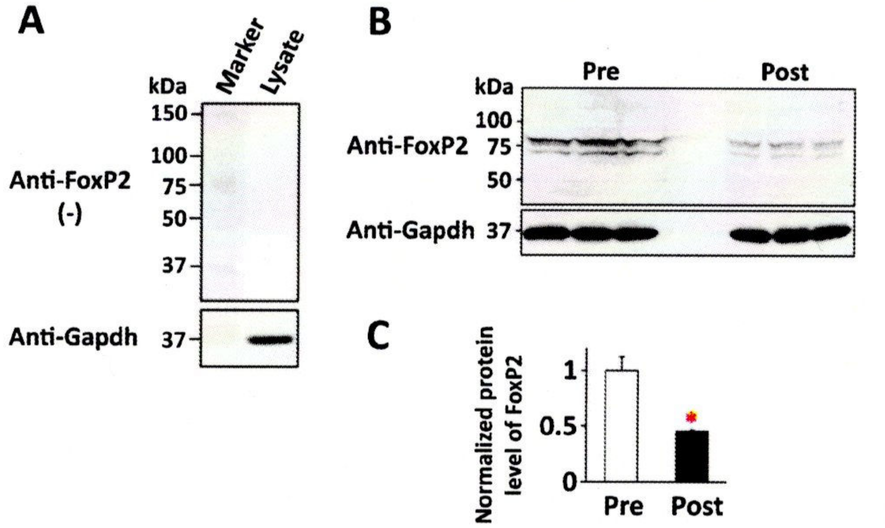
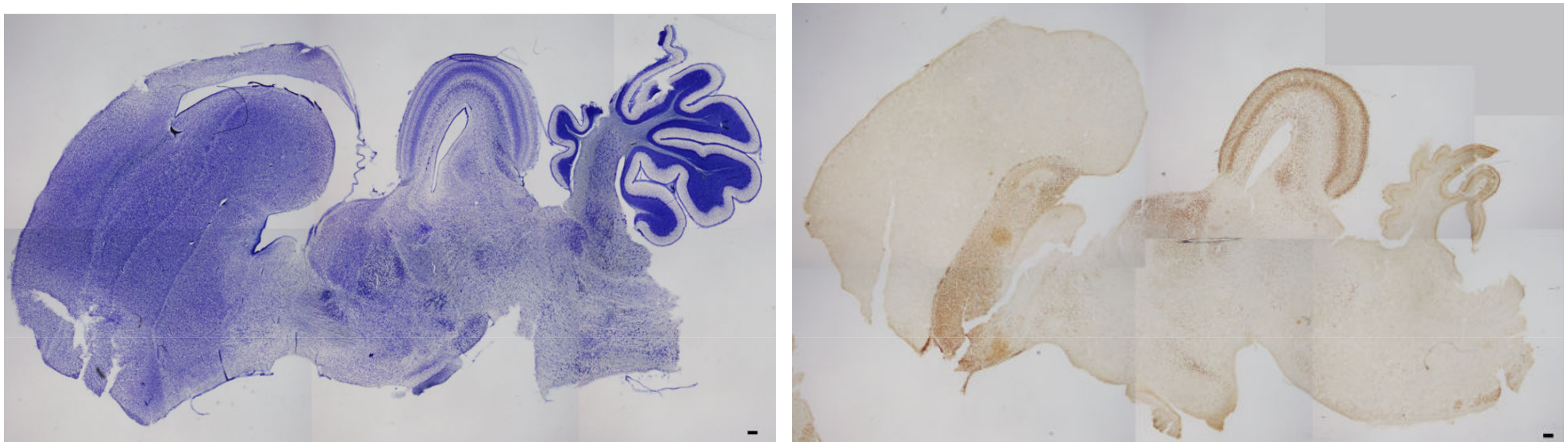


Fig. 2. FoxP2 protein levels in the midbrain of pre- and post-hatched chicks. A, Western blotting without the anti-FoxP2 primary antibody Ab1307 of the whole midbrain extract from a post-hatched chick. Note that FoxP2 protein was not detected. anti-GAPDH antibody was used as a loading control. B, Western blotting of the whole midbrain extracts from three chicks in each group. FoxP2 protein was detected using Ab1307. anti-GAPDH antibody was used as a loading control. C, Band densitometry for FoxP2 normalized to corresponding GAPDH intensity in B. Values are means ± SEM. n = 3 for each group, $^*p < 0.05$ (analyzed by unpaired *t*-test).

13. 孵化後ヒヨコ脳切片の染色像



Tissue preparation: The sperm eggs of Rhode island Red were incubated at 38 ℃ and 70% of humidity for 18/19 and 22 days. A chick was anesthetized with Somnopentyl , perfused and fixed with 4 % PFA / 0.1M PB. Cryostat sagittal brain sections (50 μm thikness) were rinsed in PBS, incubated in blocking buffer (BB), reacted with ac1307 anti-human FOXP2 antibody.IHC(ABC-DAB):The brain sections were incubated in 0.3%% hydrogen peroxide -0.3%TX, rinsed in PBS , incubated in BB, reacted with ac 1307Foxp2 antibody. then washed in PBS, incubated in biotinylated –anti goat antibody, washed in PBS, reacted with streptavidin-HRP, rinsed in Tris buffer, reacted with 0.05% DAB-0.001/ 0.05% hydrogen peroxide.

14. ニワトリ有精卵のふ化前後の胚中脳での FoxP2免疫感受性細胞の分布

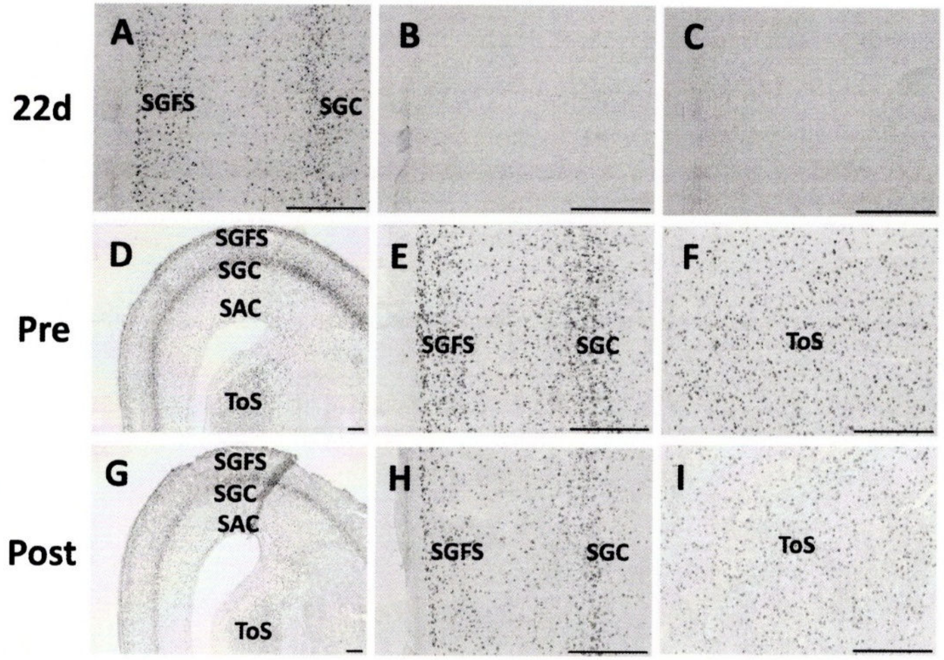


Fig. 3. Immunohistochemistry of FoxP2-ir cells in the midbrain of pre- and post-hatched chicks. A–C, Photomicrographs of a 22-d chick midbrain by FoxP2 immunostaining with Ab1307 (A), without Ab1307 (B), and with a mixture of Ab1307 and FOXP2 blocking peptide (C). D–F, Photomicrographs of a pre-hatched chick midbrain by FoxP2 immunostaining with Ab1307 (D); E, magnified image of (D) in the SGFS and SGC; F, magnified image of (D) in the ToS region. G–I, Photomicrographs of the midbrain in a post-hatched chick by FoxP2 immunostaining with Ab1307 (G); H, magnified image of (G) in the SGFS and SGC; I, magnified image of (G) in the ToS region. Scale bar = 200 μm. SAC: stratum album centrale of the optic tectum; SGC: stratum gris centrale of the optic tectum; SGFS: stratum gris fibrosome of the optic tectum; ToS; torus semicircularis.

15. ニワトリ有精卵のふ化前後の胚中脳領域でのFoxP2免疫感受性細胞数の棒グラフ

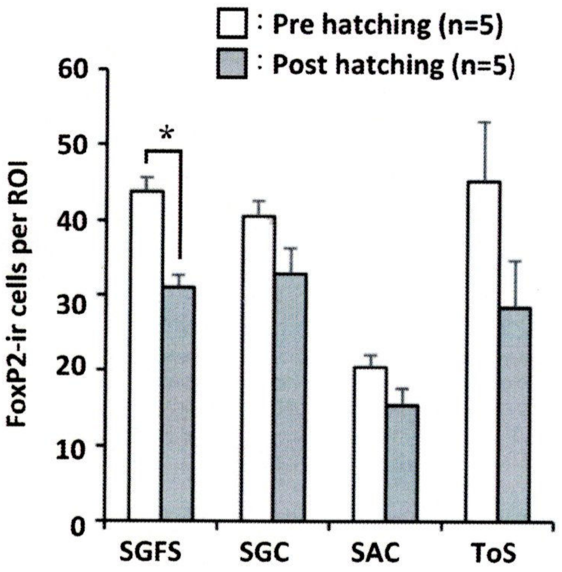
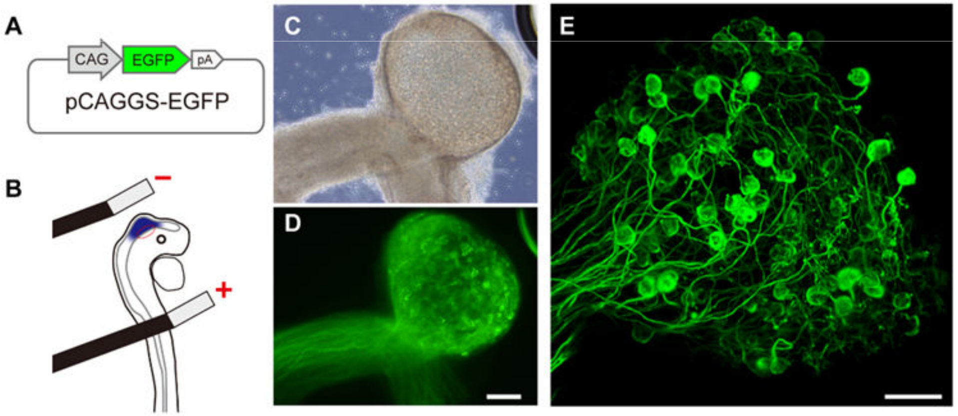


Fig. 4. Numbers of Fox *P*2-ir cells in the ROI at each subregion in pre- and post-hatched chicks. Values are mean ± SEM. n = 5 for each group, $^*p < 0.05$ (analyzed by unpaired *t*-test). See Fig. 3 for details.

In the SGFS, including the most lateral layer, the numbers of FoxP2-ir cells significantly decreased after hatching ($p < 0.05$) compared with the numbers before hatching. Although the number of FoxP2-ir cells in other regions of post-hatched chicks tended to be lower than those of pre-hatched chicks, there were no significant differences in the numbers of FoxP2-ir cell at the SGC, SAC, and ToS between pre- and post-hatched chicks (Fig. 4).

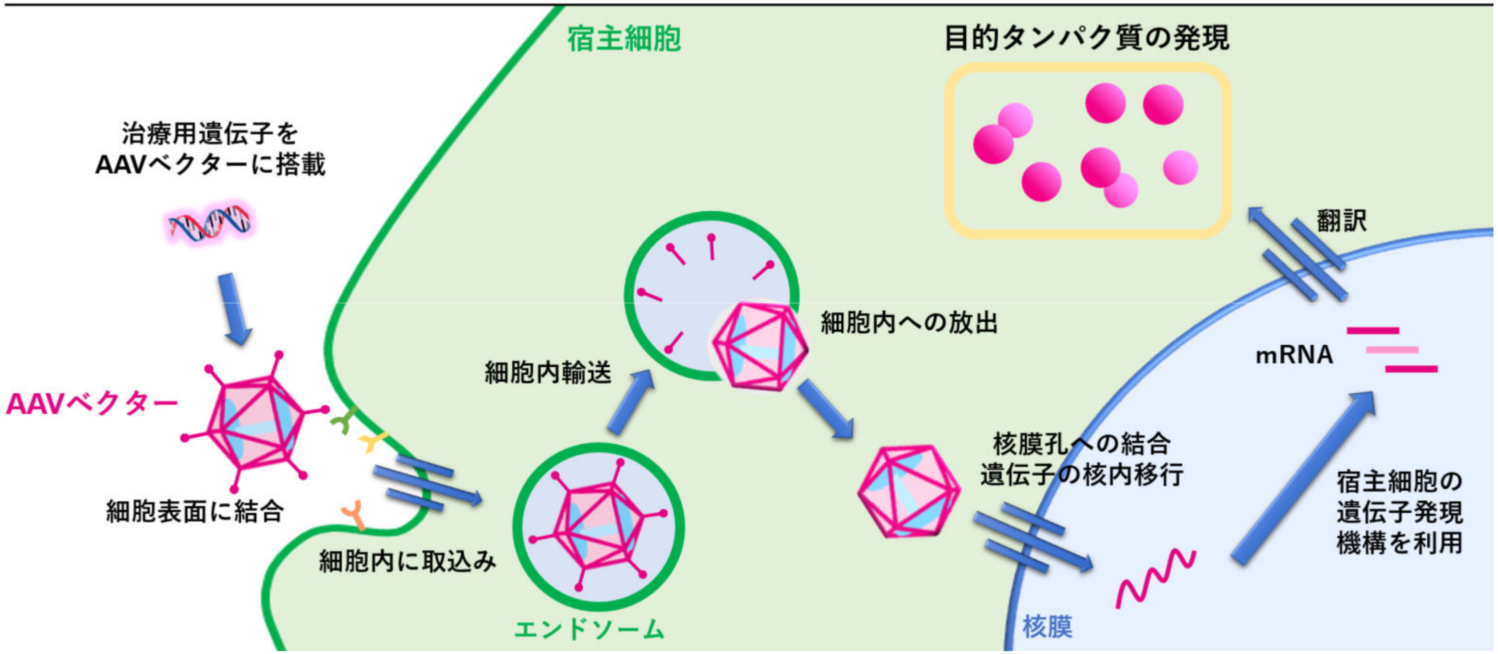
16. Progress report

Pj1; ニワトリ初期胚中脳に、ウズラQFoxP2組み組込みp-EGFDNAを電気穿孔PE法で導入して中脳でのFoxP2遺伝子発現を調べ、孵化後のヒヨコ鳴き声との関係を研究する。
Pj2; ニワトリ初期胚中脳に、ニワトリFoxP2 siRNA/shDNA組込みベクターをPE法で導入して中脳でのFoxP2遺伝子発現を調べ、孵化後のヒヨコ鳴き声との関係を研究する。
（ Pj3; ニワトリ初期胚中脳に、ウズラQFoxP2組み込みAAV又ニワトリFoxP2shDNA組込みEGF-AAVとウイルス粒子感染法で導入して中脳でのFoxP2遺伝子発現を調べ、孵化後のヒヨコ鳴き声との関係を研究する。）



R.Egawa et al(2013). Optogenetic Probing and Manipulation of the Calyx-Type Presynaptic Terminal in the Embryonic Chick Ciliary Ganglion, PLoS ONE 8(3): e59179

17.アデノ随伴ウイルス（AAV）ベクターによる遺伝子導入



AAVベクターの作用機序

18. ヒトの会話と脳神経活動 Y. Boubeneec, Nature vol.626, 2024,485-486

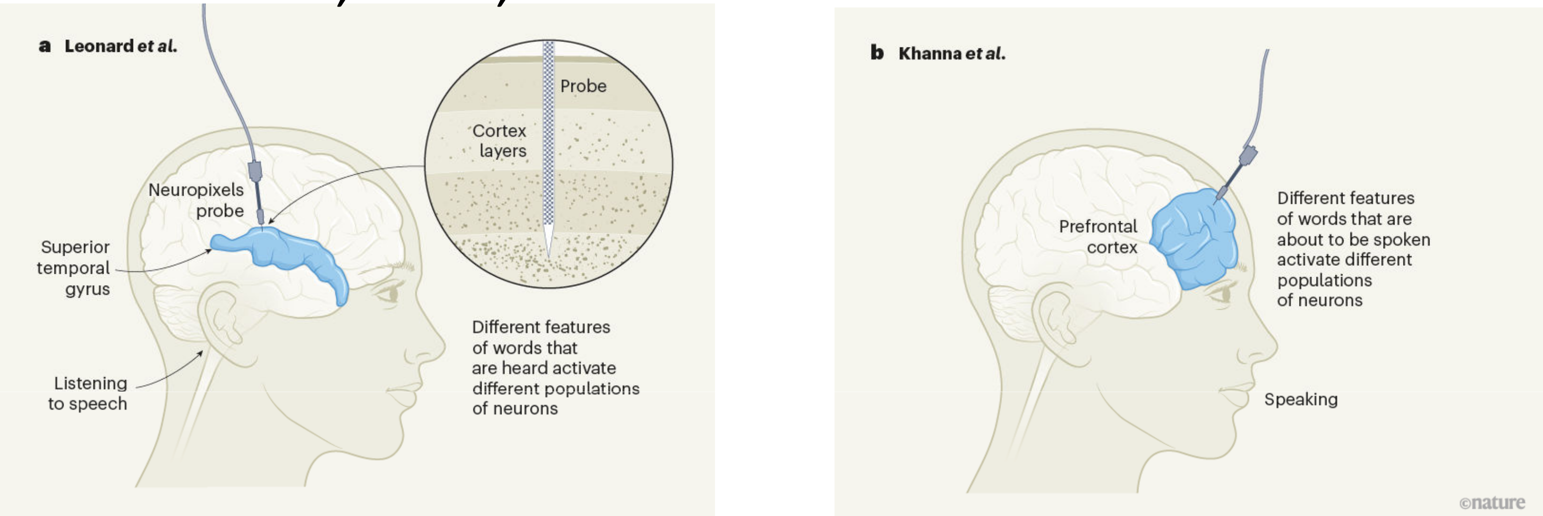
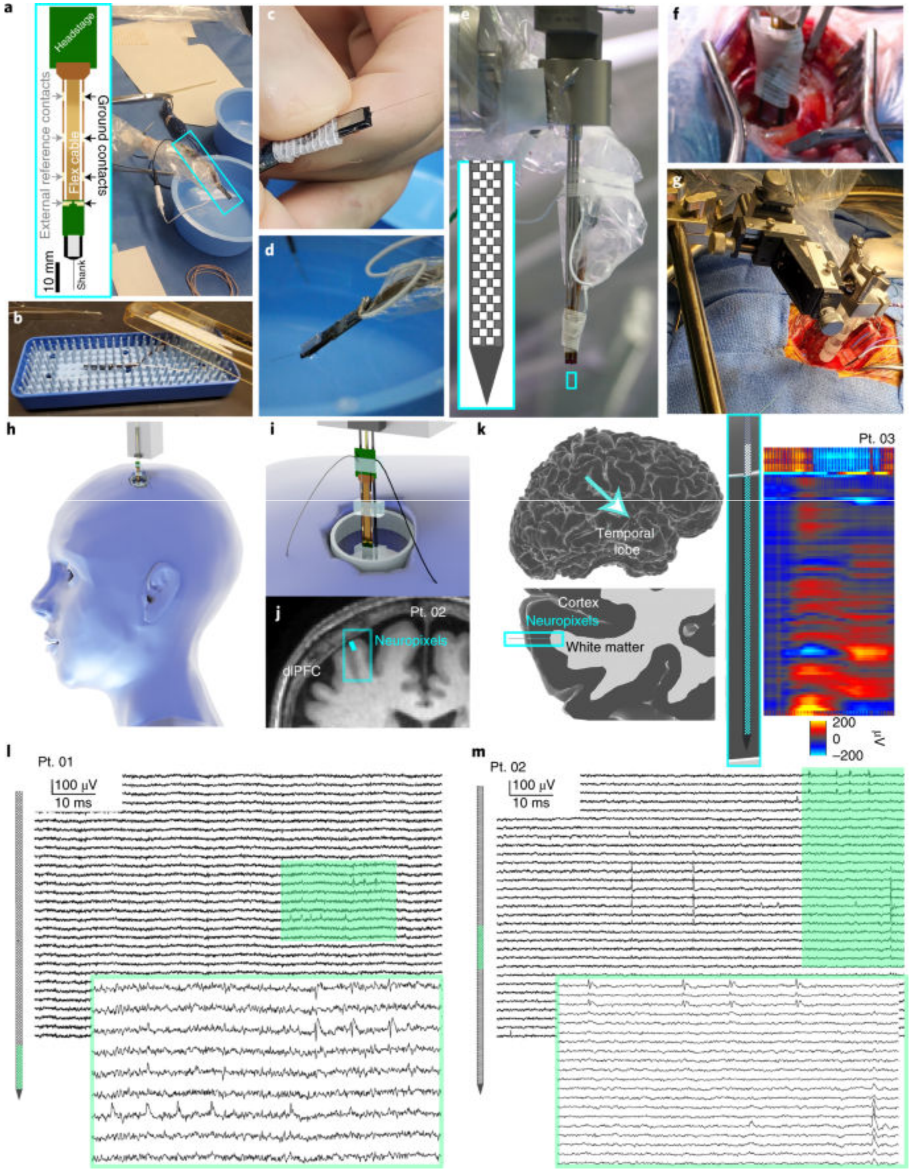


Figure 1 | **Recording** the **activity of neurons** involved in **speech processing**. a, **Leonard et al**.1 used an intracranial probe called **Neuropixels** to measure **the activity of single neurons** in the **superior temporal gyrus**, a region of the brain's auditory cortex that is involved in processing speech sounds, while participants listened to speech. b, **Khanna et al.2** used the same approach to measure neuronal activity in the **prefrontal cortex**, a brain region that is involved in word planning, while participants were speaking or listening to speech. Both teams found that **single neurons** are tuned to particular features of speech, including the sounds or the positions of **phoneme**s (the smallest units of speech) in a **word**.

19.Neuropixels ? (A.C. Paulk et al. Nature Neurosci. (2022) 25,252



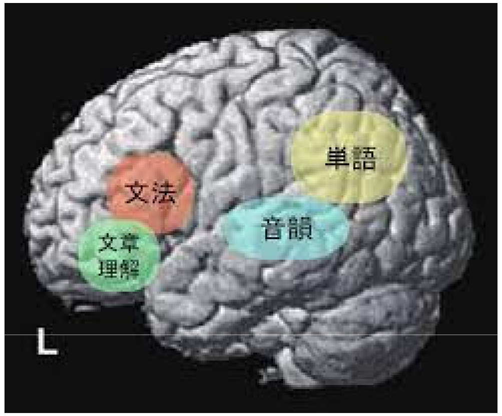
a–d, **Diagram of the Neuropixels 1.0-S probe with a headstage and the ground and reference pads indicated** (outlined in cyan, left) and preparation in a sterile field, with the probe outlined in cyan (a), set up before electrode insertion, b), handling and connecting to wires and visual inspection (c) and testing in saline (d). e, Electrode attached to three sterile stylets on the ROSA robot for insertion. f, Electrode inserted into the dlPFC through a burr hole using the ROSA robot. g, Electrode inserted into the lateral temporal cortex. h, 3D model of the DBS burr hole location with a model of the Neuropixels probe. i, Location zoomed-in view on the 3D view with the gray wire the reference and the black wire the ground. j, With the putative Neuropixels location overlaid on the preoperative MRI (top) during one DBS case, which was mapped based on the implanted location of the DBS electrode (and the burr hole) and the angles of the Neuropixels probe based on the dimensions of the holder and burr hole as well as the closest visible cortical gyrus. k, Putative location and likely depth of the electrode in an open craniotomy case for epilepsy surgery in the lateral temporal cortex (left two columns), with the depth informed by the electrophysiology, where the LFP shows a clear difference between superficial electrodes and deeper contacts, as highlighted here in a color scale indicating voltage. l, m, Example recording from Pt. 01 and Pt. 02 in the dlPFC across multiple channels, with APs shown extending across multiple channels. The light green filled-in box in the background traces are then expanded in the green-outlined voltage traces in the foreground. In a, e, j and k: cyan rectangles are highlighting the location of the Neuropixels probe.

20. 言葉と脳→言語野の発見

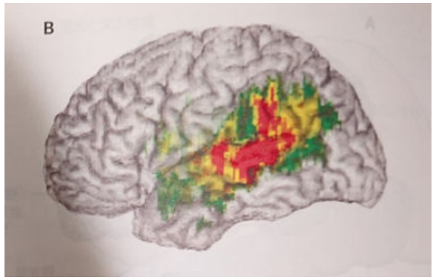
- 言語障害者の脳解剖

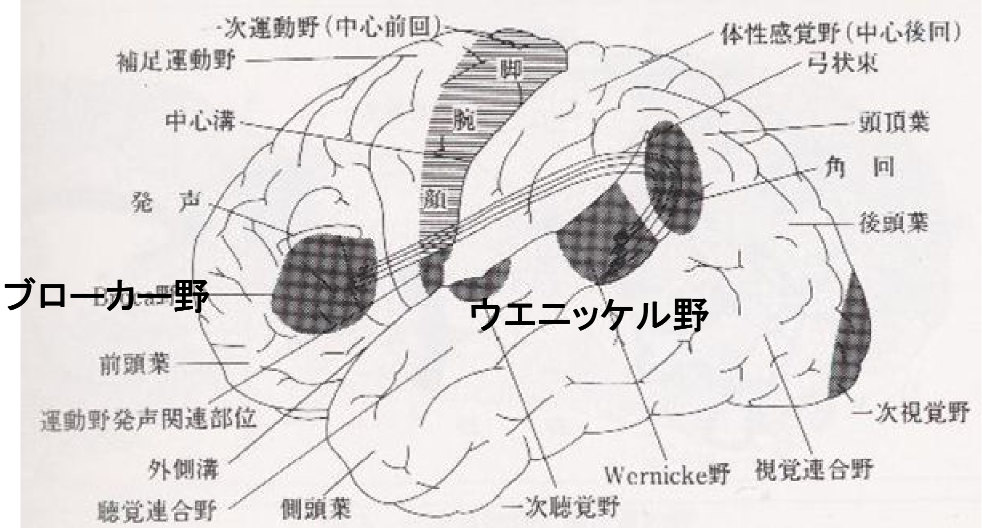
ブロカー野

タンの脳



ウェニッケル野





21. 動物言語学　シジュウカラに言語の核：2語を一つにまとめる力を確認　(T. Suzuki and Y. Matsumoto, Nature communication. (2022) 13:5605)　京大白眉研　日本語版

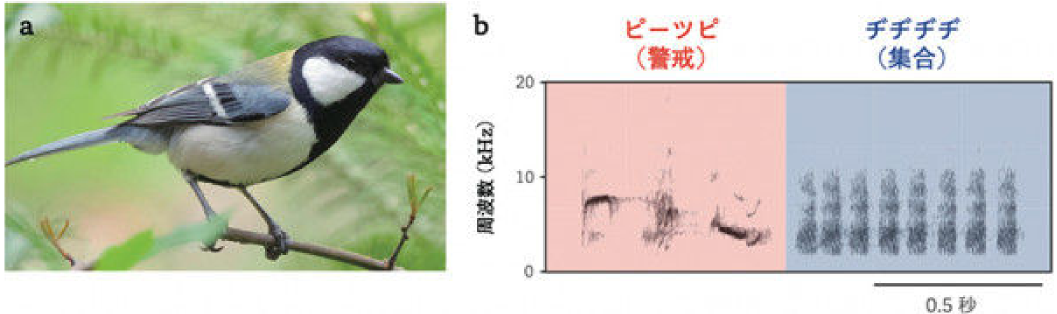


図1：シジュウカラ（a）。他個体の発した2つの異なる鳴き声の連なりを1つのまとまりと認識できる（b）

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→東大准教授　鈴木俊貴

図 2．シジュウカラに併合の力がある場合の結果の予想。（a）１羽が「ピーツピ・ヂヂヂヂ」と連ねて発した時には適切なメッセージ（警戒しながら集まれ）を解読し、天敵（モズ）を追い払うが、（b）２羽が別々に鳴き声を発した時には追い払う行動を示さないと予想される。

22.動物言語学　続き

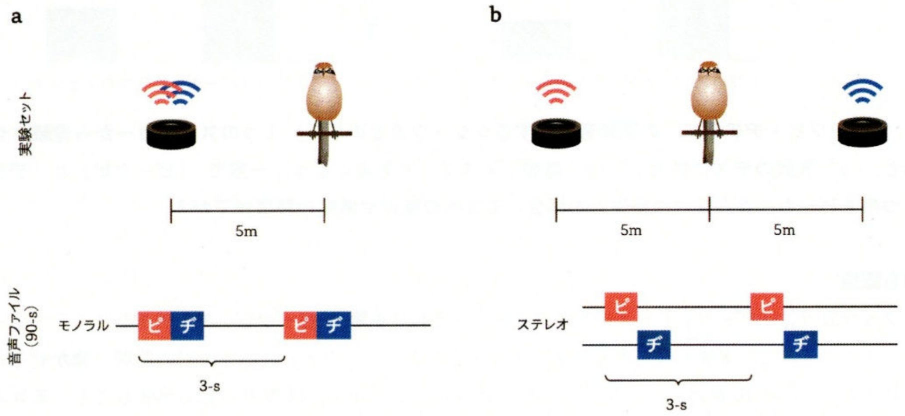

図３．実験方法。「ピーツピ」、「ヂヂヂヂ」の２音を１つのスピーカーから連続させて聴かせる場合（a）と、それと同じタイミングで２つのスピーカーから分けて聴かせる場合（b）とで、シジュウカラの行動を比較した。音声再生と同時にスピーカーから５ｍの距離に天敵のモズの剥製を設置して、それに対するシジュウカラの追い払い行動（モズへの接近と威嚇ディスプレイ）を測定しました。

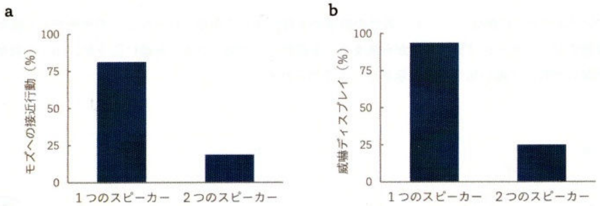

図４．「ピーツピ・ヂヂヂヂ」の連続音に対するシジュウカラの反応。１つのスピーカーから連続させて再生すると、(a）天敵のモズに接近し、(b）威嚇ディスプレイをおこなう。一方で、「ピーツピ」と「ヂヂヂヂ」を２つのスピーカーから分けて再生した場合、モズへの接近や威嚇の頻度が下がる。

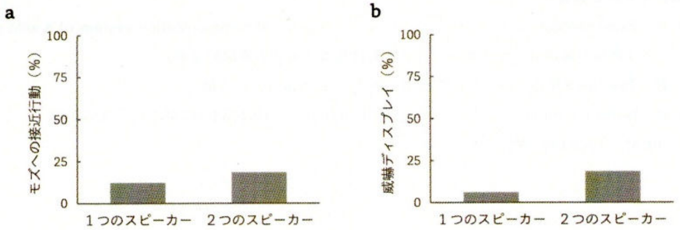

図５．語順を逆転させた「ヂヂヂヂ・ピーツピ」の連続音に対するシジュウカラの反応。１つのスピーカーから連続させて再生しても、２つのスピーカーから再生しても、(a）天敵への接近や、(b）威嚇ディスプレイはほぼみられない。

23. Brain map of human and bird related to vocalization