Sound processing in the auditory-cortex homologue of songbirds: Functional organization and developmental issues

I. George, H. Cousillas, B. Vernier, J.P. Richard, L. Henry, M. Mathelier, T. Lengagne, M. Hausberger *

UMR CNRS 6552, Université Rennes 1, Campus de Beaulieu, F 35042 – Rennes Cedex, France

Abstract

Recent literature on the Field L of songbirds, showing that some neurons present a clear selectivity towards complex sounds, especially conspecific songs, is reviewed. Furthermore, studies on European starlings have revealed a complex functional organization in this central auditory area, with subareas exhibiting different response features. Interestingly, both the functional organization and the neuronal specialization can be drastically affected by early deprivation, clearly showing the existence of a developmental plasticity. Some recovery seems to remain possible at later stages, and social factors may be involved.

© 2005 Published by Elsevier Ltd.

Keywords: Field L; Functional organization; Birdsong; Developmental plasticity

1. Introduction

Marler [45] has been one of the first authors to compare birdsong learning to language development. Based on Lenneberg [36] and Marler’s [45] studies, a convergent model of development has been proposed, with critical periods for learning (“sensitive periods”), auditory feedback requirement [32,33], and periods of practice before the production of the adult form of vocalizations (namely babbling in humans, and subsong in birds). This paradigm has led to the development of both neuroethological studies on birdsong, and behavioural studies on song learning [34,48].

In the 1970s, Nottebohm and colleagues have developed a research strategy that has led to the description of a set of discrete nuclei and their projections, now called the song system (Fig. 1A). Two main pathways have been first described: one involved in the production of song in adult birds, and the other involved in learning [4,46]. Along development, many changes can be observed in these pathways: modifications of synaptic density, changes in connections, and neurogenesis [47]. For example, the vocal control nucleus HVC and the nucleus robustus arcopallii (RA) increase in size after hatching, in relation with an increase in the number of cells, in their size, and in their connections [35].

Whereas the brain nuclei involved in song learning and production (i.e. the motor pathway) have drawn early attention, the cerebral pathway involved in the auditory perception of the vocalizations have only been recently described. Along this pathway, the Field L, located in the forebrain, is the main central auditory area, and is homologous to the mammalian auditory cortex. At present, only a few studies have investigated sound processing in this nucleus (see below). In fact, many studies have focused on motor nuclei such as the HVC, the Area X and the nucleus lateralis magnocellularis nidopallii anterioris (LMAN), where auditory neurons have been found. These neurons appear, at least in zebra finches, to be selective for...
the bird’s own song [15,41]. This selectivity seems to appear during the process of song learning, and to therefore depend on experience [16,54].

Based on these studies, Margoliash [44] has proposed a hierarchical organization of song processing, with the nucleus HVC at its top. However, recent studies have suggested that a complex processing may already occur at the level of the Field L, with neurons showing a clear selectivity towards precise parameters of conspecific songs. Given that the Field L is at the interface between the thalamic level (nucleus ovoidalis), where frequency processing has been well described, and the song system, an important issue is to understand how song is processed in this area, and whether functional studies could confirm its organization. Moreover, whereas developmental plasticity seems to occur in the sensorimotor nuclei of the song system, nothing is known about possible developmental issues in the Field L.

Here, we provide evidence for a complex spatial organization and song processing in the Field L, and, based on our studies in European starlings, we describe its functional organization both in adults and in animals early deprived...
of experience with adult song. We also question the importance of social influences on brain development. Several studies have shown that social stimulations can delay sensitive periods [2], enable learning of allo-specific vocalizations (i.e. vocalizations from another species), and induce exceptional learning, in relation to an unexpected plasticity [49].

European starlings are an interesting model because they have a variety of songs with different social implications: class-I universal whistles, which are species-specific, class-II individual-specificwhistles, and class-III warbling, which is a mainly individual-specific, modulated song [26]. Starlings also greatly depend on social conditions for learning: they learn badly from songs that are broadcast through loudspeakers [8], they share songs according to social bonds [25,26], and they can mimic human voice if interactions with the caretaker are intense [59]. Moreover, Poirier et al. [50] have recently shown that social influences can either stimulate or inhibit song learning: young starlings raised with an adult in aviaries learned mainly from one another, whereas inexperienced birds raised in pairs did not learn adult songs that were merely heard through loudspeakers while sharing with each other.

The first part of this paper reviews the recent literature on the Field L of songbirds, and the second part deals with developmental issues, both in terms of auditory and social experience.

2. Functional organization of the Field L: a review

2.1. General findings

Since the first definition of Rose [53], who has described the Field L as an area located in the caudo-medial neostriatum of birds, and composed of small densely stained neurons, descriptions have changed and the knowledge of this primary auditory area has been refined. Karten [30] has defined the Field L as a telencephalic area receiving afferences from the nucleus ovoidalis, and sending afferences to higher centres. Based on the afferences from the nucleus ovoidalis, the Field L has been divided into three subdivisions called L1, L2, and L3 [3]. More recently, based on cytoarchitectonic findings, five subcentres have been defined: L1, L2a, L2b and L3 [17,44]. Projections to and from these different areas (see Fig. 1) have been described [17,18,59].

Few studies have related Field L neuroanatomy and histology to functional properties [6,37,40]. The Field L is generally considered as a mere auditory relay between peripheral auditory centres and integrated sensorimotor nuclei such as HVC [44]. Early findings seemed to confirm this status, as a tonotopy representative of frequency distribution in the cochlea had been described by Leppelsack [37] and Zaretzky and Konishi [62]. Although Leppelsack and Vogt [38] have mentioned the existence of neurons selective for species-specific sounds in starlings, only recently has more evidence emerged: there has been reports of responses to frequency sweeps rather than pure tones in chickens [27,28], to harmonics in zebra finches [40] and to learned allo-specific sounds in mynahs [57]. Grace et al. [21] have also found that Field L neurons tend to respond more to conspecifics songs than to artificial sounds exhibiting similar acoustic properties.

Finally, using complex stimuli containing harmonics, Lim and Kim [40] have shown that the three main subdivisions of the zebra finch’s Field L were functionally distinct: L1 and L3 neurons showed more activation and inhibition in response to complex harmonics, whereas L2 neurons responded equally to pure tones and to harmonics.

2.2. “Mapping studies” in starlings

Capsius and Leppelsack [6] have used a thorough method of “mapping”, using pure tones, to systematically record neuronal responses over a whole area. Penetrations were made at 200 µm intervals, and recordings were performed every 100 µm in depth. They have found that the spatial distribution of the responses to frequencies was not random, and that they were able to define eight functional subareas: NA-L, NA4, NA3, NA2A, NA2B, NA2C, HVA (auditory hyperstriatum ventrale), and AP (auditory paleostriatum). These different subdivisions correspond to different gradients of tonotopy and to different response properties. According to Capsius and Leppelsack [6], NA-L covers the anatomically defined Field L [53], and the major part of L1, the complete L2, and parts of L3 are located within NA-L. The area NA2A does not seem to correspond to any previously described functional area of the Field L. The area NA3 lies caudal to NA-L and includes the caudal part of L3. The area NA4, an area caudo-dorsal to NA-L, represents the auditory region in the direction of the HVC and possibly corresponds to the “shelf” [31].

As complex sound coding seems to already arise at the level of the Field L [21], investigating responses to natural sounds [56] appeared to be the next necessary step. First, using single cell recordings in awake-restrained adult male starlings, we observed that 80% of the 320 recorded neurons only responded to precise parts of species-specific sounds [24]. A backward correlation method [51] allowed us to visualize which elements of the stimuli broadcast were more likely to evoke activation or inhibition in the neurons concerned (Fig. 1D). Interestingly, “key” elements seemed to emerge. These elements corresponded to features that play an important role in the discrimination of whistle types [23]. Furthermore, neighbouring neurons often showed similar preferences for combinations of parameters (Fig. 1E). Some spatial organization therefore might exist.

Multiunit recordings using Leppelsack’s group mapping method (using 200 µm intervals in both penetration and depth) gave us further insight into the functional organization of the Field L. Using a set of natural sounds, we have
mapped the neuronal responses in different birds (mean = 480 recording sites per bird, \( N = 8 \) birds), and we have found that: (1) areas of activation for a given whistle type are consistent, (2) slight individual differences exist, and (3) Capsius and Leppelsack’s [6] description of Field L in starlings could be refined (Fig. 2). Indeed, we could characterize five functional subareas, based on the responses to natural sounds, and their spatial distribution was consistent: (1) with Capsius and Leppelsack’s [6] description (Fig. 3), and (2) between two groups of starlings exposed to different sets of stimuli (data not shown). Four of the described areas appeared to correspond to the four zones generally described in the histological data of other species [17]. A complex organization therefore emerges, with a main central area characterized by a “classical” tonotopic gradient and showing relatively “basic” responses to sounds, surrounded by more specialized subareas. These findings are consistent with Lim and Kim’s [40] results indicating a greater specialization in L1 and L3. Finally, histological data suggest that these areas could have connections with the HVC (or the shelf). This would imply that there may be a “pre-processing” of relevant signals, which would then be integrated in potential motor responses.

2.3. Processing of conspecific songs in awake and anaesthetized starlings

We have investigated neuronal responses to well-known song categories that have different social implications.
Systematic recordings were performed, and single neuron responses were selected [19]. Recordings were made in six wild-caught adult males that were first tested while awake and restrained, and then while anaesthetized with urethane. 44.8% of the recorded neurons responded to at least 1 stimulus when the birds were awake, and 50% when they were anaesthetized. Neurons were divided into two groups: “A neurons” (33.4% of the responsive neurons when the birds were awake, and 21.2% when they were anaesthetized) responded to only 1 stimulus, and “B neurons” (66.6% and 78.8% respectively) responded to more than 1 stimulus.

Whatever situation was considered (awake or anaesthetized), more than 80% of the responsive neurons responded to individual-specific songs (class-II whistles and class-III warbling), including songs of an unfamiliar bird. Under anaesthesia, the proportion of responses to artificial, non-specific sounds was higher than when the birds were awake. Neurons therefore appeared to respond mainly to signals that required a mere frequency processing when the birds were anaesthetized, whereas they seemed to respond mainly to sounds requiring a finer and more complex processing when the birds were awake.

The spatial distribution of the responses was consistent with earlier findings, and confirmed this last trend: in awake birds, most responses were observed in NA3, and to a lesser extent in NA2a, while under anaesthesia, most responses were located in NAL. Finally, the most “specialized” neurons (the “A neurons”, responding to only 1 stimulus) tended to be found in NA3 and NA2a, whereas “B neurons” (responding to more than 1 stimulus) were located mostly in NAL. This was especially clear in anaesthetized birds (Fig. 4).

Our results are therefore consistent with previous findings: they again show a central area (NAL, which contains L2) involved in frequency processing, even under anaesthesia, and surrounded by more specialized regions that are mainly activated when the birds are awake.

3. Developmental issues: an experimental study

Zhang et al.’s detailed studies [60,61] have revealed that early acoustic environment influences the functional development of rats’ auditory cortex. Both the functional organization and the neuronal selectivity have been shown to depend on early auditory experience. More recently, Chang and Merzenich [11] have also shown that environmental noise retards auditory cortical development in rats. Given the ability of songbirds to learn their songs, these animals are a particularly appropriate model to study early plasticity.

The recently acquired knowledge on the functional organization and on the neuronal specialization in the Field L makes it interesting to see how this is set up, and what effect auditory deprivation of adult song during development can have on the functional organization and on the specialization of Field L in European starlings. To our knowledge, the effect of early experience on the functional organization of auditory areas has never been investigated in songbirds yet.

Fig. 4. Representative maps showing the distribution of the responses to a given stimulus (left panel) and of A and B neurons (right panel) over the functional subareas of the Field L defined by Capsius and Leppelsack [6], in an awake (top panel) and then anesthetized (bottom panel) wild-caught bird (modified from [20]). For each map, the caudo-rostral position (in μm) of the recording sites is on the x-axis, and the depth (in μm) on the y-axis. Each rectangle corresponds to one recording site. In the left panel, the number of responses during a given stimulus (here a class-III warbling motif) is represented by a grey scale going from white for no response to black for 10 responses or more. V: Ventricule.
Hand-raised starlings develop abnormal songs when they cannot hear adult songs, and they do not learn well from mere playbacks [1,8,9]. However, some structural aspects of songs appear to be better organized when animals are raised in pairs or in groups of young birds [10]. The question of a possible recovery, and of the conditions required for it, has thus been raised. Is the effect of early deprivation a permanent effect? Could later exposure to song and/or direct contact with adults increase the chances of a recovery? We have addressed these questions by investigating: (1) the effects of auditory deprivation of adult song during development on neuronal responses once the birds have become adults [12], and (2) the possible recovery of early-deprived animals housed with singing adults after 1 or 2 years of deprivation.

3.1. Materials and methods
3.1.1. Subjects
Four conditions were used (Fig. 5):
• Control birds (N = 4) were wild-caught adult starlings (Departmental direction of the veterinary services of Ille-et-Vilaine agreement, licence number 005283).
• Group-I birds (N = 4) were hand-raised starlings that had been kept as a group of young birds isolated from adults during 2 years, until the electrophysiological recordings were performed.
• Group-II (N = 4 males and 3 females) and group-III birds (N = 3 males and 4 females) were hand-raised starlings that had been kept as a group of young birds isolated from adults during 2 years, until the electrophysiological recordings were performed.
• Group-II (N = 4 males and 3 females) and group-III birds (N = 3 males and 4 females) were hand-raised starlings that had been kept as a group of young birds during 2 and 1 year respectively, until they were placed in an aviary with four wild-caught adult birds (2 males and 2 females) for 1 year.

Fig. 5. Schematic representation of the protocol used for the developmental experiments. The control group (not shown here) was composed of wild-caught starlings. All experimental birds were hand raised. Group-I birds (top arrow) were kept isolated from adults during 2 years. Group-II birds (bottom arrow) were kept isolated from adults during 2 years, and were then placed in an aviary with adults for 1 year. Group-III birds (middle arrow) were kept isolated from adults during 1 year, and were then placed in an aviary with adults for 1 year.

Fig. 6. Examples of sonograms of some of the stimuli used in our experiments. Class-I whistles are sung by all the males. Class-II whistles and class-III warbling are individual-specific songs. The stimulus set contained familiar and unfamiliar stimuli.
3.1.2. Stimuli

Twelve to 21 stimuli were used. Most of these stimuli were species-specific whistles that consisted of motifs taken from either an unfamiliar, a familiar or the birds’ own song (Fig. 6). Stimuli were filtered, and analysed using an Amiga 3000 programmed for sound analysis and synthesis [52]. The whole stimulus set was 10 s long. The intervals between stimuli were 300 ms. The stimulus set was presented in an anechoic, sound-attenuating chamber, through a loudspeaker placed 20 cm in front of the bird’s head. The maximum sound pressure at the bird’s ears was 60 dB SPL (re 20 μP), measured by a 1/2 microphone (LEA S.S.T.4S). The stimulus set was repeated 10 times at each recording site.

3.1.3. Recordings

Before the neurophysiological experiments, a stainless steel well was implanted stereotaxically on the bird’s skull, under halothane anaesthesia. The implant was located precisely with reference to the bifurcation of the sagittal sinus: 2.5 mm rostral, and 1 mm in the left hemisphere. These values were the coordinates of the centre of the parasagittal recording plane. After surgery, birds were allowed to recover for three days. During this time, and between experimental sessions, they were kept in cages with conspecífics. During the experiments, the well was used for fixation of the head, and as an indifferent electrode (for further details, see [6]).

Electrodes were made by Frederick Haer and Co. They consisted of a tungsten wire insulated by epoxyite, with a fine tip (angle 10–15°). Electrode impedance was in the range of 2–4 MΩ. An Amiga 3000 computer was used to record action potentials. A custom-made analogous/digital card was used to digitize multicellular recordings, and all the action potentials crossing the threshold of a computed window discriminator were counted.

In order to compare exactly the same functional areas in experimental and wild-caught birds, the recording plane was set precisely at the same location for all the birds. Recordings were performed at 30–40 sites along the path of one electrode penetration. A single recording session usually lasted about 3 h. During recording sessions, the birds were awake, and they were kept in a jacket in order to limit their movements. One recording plane was made in the brain of each bird. This plane was a parasagittal plane, located 1 mm in the left hemisphere with reference to the sagittal midline. Penetrations within a single recording plane were placed at intervals of 200 μm. For each penetration, recordings started 600 μm below the brain surface, at a site that gave no auditory response, and continued until 4000 μm below the brain surface, where auditory responses were no longer detectable. The recording plane was considered complete when no response was obtained in both outermost penetrations (for further details, see [6]).

3.2. Data analysis

Experimental data were recorded with a temporal resolution of 0.1 ms. Spontaneous activity was calculated using the activity recorded during the 100 ms preceding the beginning of each auditory stimulus. Then, for all the recording sites and all the stimuli, peri-stimulus time histograms (PSTHs) were calculated using a temporal resolution of 2 ms. Student-Fisher t tests were used to determine the significance level of the response (activation, p < 0.02; inhibition, p < 0.05). We chose a probability level of 0.05 for inhibition because, given the low number of spikes/2 ms during spontaneous activity (0.4–0.7 spikes/2 ms), the contrast between spontaneous activity and inhibition was difficult to confirm statistically.

3.3. Results

Neuronal activity was recorded at more than 240 sites per bird. Many differences could be observed between the groups (see 3.1.1 Subjects). First, the number of auditory sites differed (Kruskal Wallis test, H = 10.9, p = 0.011). Indeed, the number of auditory sites was clearly higher in totally-deprived animals (group I) than in wild-caught birds (control; Mann-Whitney U test, U = 0, p = 0.014) and than in experimental animals that were later housed with adults (Mann-Whitney U tests, group II: U = 0, p = 0.007, group III: U = 0, p = 0.014; Fig. 7A). However, group-II and -III birds did not differ from wild-caught animals (Mann-Whitney U test, U = 6 and 19 respectively, p = 0.17 and p = 0.88). Differences could also be observed between the other experimental groups and the controls (Mann-Whitney U test, Groups 2 and 3/Wild: U = 0 in both cases, p = 0.0015 and 0.027 respectively, Group 2/Group 3: U = 5, p = 0.067).

![Figure 7](image-url)
Early deprivation therefore may, to some extent, be compensated by later exposure to adults. However, being deprived either during the first or during the first two years of life does not seem to really change the possibilities of recovery. Interestingly, the proportion of non-specialized neurons in group-II birds was even closer to that of controls, although they had been deprived of adult song for a longer time period than group-III birds.

From a behavioural point of view, none of the experimental animals reached the singing performance of the control birds, and none of them copied their adult tutor (Henry et al., in prep.). At this stage, we do not know whether early deprivation induced deficits in learning abilities, or if this may be due to social separation between adults and younger animals, as shown in previous studies [50]. In our study, when experimental animals were mixed with control birds, group-III birds remained together, without any close contact with wild-caught adults, whereas adult group-II birds interacted with them.

4. Conclusion

The main central auditory area of songbirds, namely the Field L, may well be a very important “gate” to the song system. While there is increasing evidence of neuronal selectivity at high level [29,40], especially towards natural sounds and species-specific song characteristics (inflection, slope, double voice), investigations of the functional organization in different species shed light on how the system works and how it may be an important step for further processing in the nuclei involved in song production. How songs are filtered and processed in the Field L may be a prerequisite to understand the behavioural level. While both studies performed on the spatial organization of the Field L in European starlings converge in terms of the location of the subareas, and their level of specialization, further studies are needed to know how the information may transfer from one subarea to another. The central NAL area shows more “elementary” responses, in particular frequency processing (e.g. tonotopy), than the neighbouring NA3 and NA2 subareas [7,13]. It remains to be known if this central area may be a primary source of information for the other subareas. This does not seem to be the case in Grace et al.’s study in zebra finches [21], but further studies are required on this topic. This is a complex issue, especially when taking into consideration that the different areas seem to be differently affected by urethane anaesthesia [20]. Individual characteristics of song seem to be mainly processed when the birds are awake, whereas more general information, and particularly frequency parameters, seem to elicit responses when the birds are anaesthetized, especially in the central area. Further studies have revealed that there is a hemispheric specialization, with the left hemisphere preferentially processing whistled structures when the birds are awake (especially the class-II whistles involved in the recognition of individual identity), and the right hemisphere processing warbling song when the birds are awake, and nonspecific information when the birds are anaesthetized [20]. However, nothing is yet known about how this lateralization takes place during development.

Both the functional organization and the neuronal specialization are influenced by experience, as shown by our results in starlings [12]. Early deprivation of adult song has drastic effects, but partial recovery seems to be possible when the birds are brought back in avaries with wild-caught adults. Thus, while the fully deprived animals showed an enlarged auditory area, this auditory area was comparable to controls after having experienced contact with adults. Zhang et al. [60,61] have found that, in rat pups, a limited acoustic environment during the two first months of life induced abnormalities in the primary auditory cortex, including poor neuronal selectivity, degraded tonotopy, and much larger auditory area (see also [11]). Experimental rats did not present the normal developmental decrease in size of this area, contrarily to control rats. Unfortunately, the authors did not test for possible recovery. In our study, the recovery of neuronal specialization appeared to be only partial. Intriguingly, birds that had been deprived during 2 years tended to show more neuronal specialization than birds that had been deprived during only 1 year. Further studies are needed before this phenomenon can be confirmed and explained. However, observations of the social behaviour showed that the experimental birds had more interactions with the adult control birds when they were reintegrated as adults (2 years old) than when they were introduced at 1 year old. Previous experiments have shown that: (1) young birds may “neglect” the auditory information coming from adult models if they develop bonds with same-age peers [50], and (2) neuronal specialization may be influenced by social experience, even if adult song is provided [14]. It therefore seems that the relatively low level of recovery may be due more to a lack of social interaction (see also [55]) than to some physiological impairment. Further studies in which social interactions are controlled should allow us to address this issue.

References


