Effect of auditory cortex lesions on the discrimination of frequency-modulated tones in rats

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Abstract

The lateralization of functions to individual hemispheres of the mammalian brain remains, with the exception of the human brain, unresolved. The aim of this work was to investigate the ability to discriminate between falling and rising frequency-modulated (FM) stimuli in rats with unilateral or bilateral lesions of the auditory cortex (AC). Using an avoidance conditioning procedure, thirsty rats were trained to drink in the presence of a rising FM tone and to stop drinking when a falling FM tone was presented. Rats with a lesion of the AC were able to learn to discriminate between rising and falling FM tones; however, they performed significantly worse than did control rats. A greater deficit in the ability to discriminate the direction of frequency modulation was observed in rats with a right or bilateral AC lesion. The discrimination performance (DP) in these rats was significantly worse than the DP in rats with a left AC lesion. Animals with a right or bilateral AC lesion improved their DP mainly by recognizing the pitch at the beginning of the stimuli. The lesioning of the AC in trained animals caused a significant decrease in DP, down to chance levels. Retraining resulted in a significant increase in DP in rats with a left AC lesion; animals with a right lesion improved only slightly. The results demonstrate a hemispheric asymmetry of the rat AC in the recognition of FM stimuli and indicate the dominance of the right AC in the discrimination of the direction of frequency modulation.

Introduction

The auditory cortex (AC) is involved in the processing of complex acoustic signals such as human speech and species-specific vocalizations in mammals. Speech, as well as many animal calls, consists of complex sounds that are usually frequency-modulated. It is natural therefore that frequency-modulated (FM) stimuli have been used in the investigation of AC function for a long time. The results of the study by Whitfield & Evans (1965) suggested that many neurons in the auditory cortical area AI in the cat are sensitive to the direction of frequency change. At the same time, Suga (1965) demonstrated that in the AC of bats, cells are not only sensitive to FM sweep parameters, but also that such cells are located in a region of the cortex that is functionally specialized for processing of FM signals.

In addition to electrophysiological studies, there is behavioral evidence for the role of the AC in the processing of FM stimuli, obtained in experiments utilizing an AC lesion. It was shown that cats had considerable difficulty in learning to discriminate between rising and falling FM tones and also in retaining their ability to discriminate after large bilateral lesions of the AC (Kelly & Whitfield, 1971). A sensory deficit in FM tone processing was also described in gerbils in which bilateral lesions of the AC were made (Ohl *et al.*, 1999). A lesion in the right AC markedly impaired the gerbils' ability to discriminate the direction of frequency modulation, whereas a lesion of the left AC did not result in such a deficit (Wetzel *et al.*, 1998). The hemispheric lateralization of functions in experimental animals was

the subject of several further studies, without reaching a unanimous consensus (for reviews see Vallortigara *et al.*, 1999; Rogers & Andrew, 2002).

The aim of the present study was to investigate the role of the AC in discriminating between rising and falling FM tones by comparing the discrimination performance (DP) in rats with a bilateral or unilateral lesion with the DP in control unlesioned rats. With respect to the results obtained by Wetzel et al. (1998), we expected that the rats with a right AC lesion would show a greater deficit in the discrimination of FM stimuli than those with a left AC lesion. The effect of an AC lesion on the discrimination of the direction of frequency modulation could be different between rats that start training after the cortical lesion and those trained before the lesion. In the latter case, the animals are able to use some undamaged memory traces in the brain, whereas in the former case they learn a completely new task. Therefore, we studied the DP in rats that were lesioned before or after discrimination training. In addition, we investigated the role that individual parameters of the FM tones play in the discrimination training of normal and lesioned rats. In this regard, we studied the transfer of an acquired conditional reaction in trained rats to novel stimuli.

Materials and methods

Subjects

Young-adult female pigmented rats (strain Long Evans) with no primary pathology, aged 2–3 months and weighing 180–250 g at the beginning of the experiment, were used as experimental subjects. The

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rats were housed under standard laboratory conditions, 2–3 rats in a cage, and had free access to food but were restricted in access to water. Two days before the beginning of training, the rats were completely deprived of water; afterwards, water was available only during training or testing sessions.

Three experimental groups of rats were used: (i) control rats (three normal, two left sham operated rats and two right sham operated rats); (ii) rats with an AC lesion made before the discrimination training (three rats with a lesion of the left AC, three rats with a lesion of the right AC and three rats with bilateral lesions of the AC); and (iii) rats with an AC lesion made after discrimination training (three rats with a lesion of the left AC.

Behavioral apparatus

Using an avoidance conditioning procedure, thirsty rats were trained to drink in the presence of a rising FM tone and to stop drinking when a falling FM tone was presented (paired with a mild electric shock when the response was absent). Training and testing were conducted in a rat test cage (Coulbourn, model H10-11R-TC, $50 \times 30 \times 50$ cm) placed in an anechoic, sound-proof room. The original plastic walls of the cage were replaced by grid walls with the aim of making them acoustically transparent. The cage was provided with equipment for drinking, an optical licometer (Coulbourn, model H24-01R) for automatic detection of licking and a 'shock floor' (Coulbourn, model H10-11R-TC -SF) connected to a precision-regulated shocker (Coulbourn, H13-16), which was able to generate a level-adjustable electric footshock (100-300 µA). The level of shock was individually adjusted for each animal in the first three sessions so that it could produce a reliable avoidance response consisting of backing away lightly from the spout or lifting its head from the spout. A waterspout protruded through the front side of the cage 7 cm above the floor and 50 cm from the loudspeaker, which was placed outside the cage. A video camera situated near the cage permitted visual monitoring of subject behavior during the experiment. The equipment for stimulus generation and response acquisition was based on a set-up consisting of a TDT system 3 connected to a PC. Custom-made software controlled the stimulation and recording procedure. The PC, the TDT system 3, the shocker and a video monitor were located outside the sound-proof room.

Training and testing procedure

An FM tone with a frequency linearly rising from 3 to 6 kHz was used in the initial discrimination training as a safe stimulus, and an FM tone linearly falling from 6 to 3 kHz was used as a warning stimulus. The FM tone parameters were: modulation rate, 1.33 oct./s; duration, 750 ms; intensity, 70 dB SPL (Fig. 1A). One session consisted of up to 154 trials, which were delivered under computer control in a quasirandom manner with a ratio of safe trials to warning trials of 1.8 : 1. A warning trial was never followed by another warning trial. Each trial started only if the animal was licking and lasted for 1250 ms, including the time of the stimulus presentation (750 ms) plus a response period (500 ms) when the rat's licking was detected. One session lasted about 20 min, during which time the rat consumed up to 20 mL of water and underwent, as a rule, 120–154 trials.

The cessation of licking after a warning stimulus was classified as a hit response; the cessation of licking in response to the safe stimulus was considered as a false alarm response. The hit rate (H) was calculated as a ratio of hit responses to the number of warning stimuli; the false alarm rate (F) was calculated as the ratio of false alarm

Stimuli for initial training



FIG. 1. Schemas of the acoustic stimuli used in the experiments. (A) Stimuli for the initial training: FM tones falling from 6 to 3 kHz and rising from 3 to 6 kHz with a modulation rate of 1.33 oct./s. (B–E) novel stimuli: (B) pair I: pure tones at 6 and 3 kHz; (C) pair II: pure tones at 3 and 6 kHz; (D) pair III: FM tones falling from 6 to 3 kHz and rising from 3 to 6 kHz with a modulation rate of 4 oct./s; (E) pair IV: FM tones falling from 4 to 2 kHz and rising from 4 to 8 kHz with a modulation rate of 1.33 oct./s. Solid line – warning stimulus, dotted line – safe stimulus.

responses to the number of safe stimuli, and the ability to discriminate was quantified by the discrimination performance (DP), calculated as DP = H - F and expressed as a percentage. Scores could range from 100% (perfect DP, when H reached 100% and F reached 0%) to 0% (chance performance, when the animal was unable to discriminate between the presented stimuli and the H and F values were similar). It should be noted that the performance could also be negative (H < F), in cases in which the rat stopped licking in response to safe stimuli and continued to drink during warning stimuli presentation. The significance of the difference of the DP from zero was tested in each rat for a single session using the χ^2 test. Four possible combinations of stimuli and responses (warning stimulus - hit, warning stimulus miss, safe stimulus - false alarm, safe stimulus - correct rejection) were analysed. For statistical evaluation of the changes in DP within the same group and the comparison of DP between groups, a paired and unpaired *t*-test was used, respectively.

The discrimination training for both normal rats and rats with an AC lesion lasted until the values of DP reached a steady level and further prolongation of the training period did not lead to any improvement in the DP. When the slope of the regression line constructed from the DPs of the last consecutive sessions (minimally six) did not significantly deviate from zero (P > 0.1), we considered that the DP

had reached a steady level. The final DP after training was calculated as the mean DP of the last six sessions.

When discrimination training was finished, three control rats and all rats with an AC lesion were tested using novel pairs of stimuli that were different from the initial training stimuli in some parameters. The ability to discriminate between stimuli in the novel pairs was studied with the aim of evaluating the role of the individual parameters of the FM tone (frequency at the beginning or at the end of the FM tone, the modulation rate and the direction of modulation) in the discrimination task.

Four novel pairs of test stimuli were used for discrimination: (I) pure tones with frequencies of 6 and 3 kHz – the 6-kHz tone was used as a warning stimulus and the 3-kHz tone as a safe stimulus (Fig. 1B); (II) the same pure tones (6 and 3 kHz) with reversed meaning, i.e. the 3-kHz tone was used as a warning stimulus and the 6-kHz tone as a safe stimulus (Fig. 1C); (III) FM tones rising from 3 to 6 kHz and falling from 6 to 3 kHz with a faster modulation rate than the initial FM tones used in discrimination training (4 oct./s instead of the initial 1.33 oct./s, Fig. 1D); (IV) FM tones rising and falling from the same initial frequency (falling from 4 to 2 kHz used as a warning stimulus and rising from 4 to 8 kHz used as a safe stimulus, Fig. 1E).

The novel stimuli had the same intensity (70 dB SPL) and duration (750 ms) as the initial FM tones used in discrimination training. The FM tones with a faster modulation rate than the original FM tones (novel pair III) consisted of two 250-ms FM tones separated by a silent period of 250 ms, and thus the total duration of the stimulus was also 750 ms.

The pure tones (novel pairs I and II) were used only in one test session. The modified FM tones (novel pairs III and IV) were presented in seven subsequent sessions (i.e. training with each novel pair of FM tones was conducted for 1 week). Before using another novel pair of stimuli, the animal was exposed for at least three sessions to the initial training stimuli (rising 3–6 kHz and falling 6–3 kHz FM tones) until the DP values reached the same value as after initial training.

In six rats, a unilateral lesion of the AC was made after discrimination training (in three rats a lesion of the left AC and in three rats a lesion of the right AC). Seven days after surgery, including a water deprivation period, these animals were tested as to whether they were able to discriminate the same stimuli as were used in the discrimination training. During the following 3 weeks, the rats were retrained to discriminate FM tones. In addition, the rats' ability to discriminate pure tones (novel pair I) was tested at the beginning and at the end of the retraining period.

Acoustic apparatus

The FM tones and pure tones used in the experiments as conditioned stimuli were generated by a TDT system 3 set-up, which consisted of a real-time processor (RP2), two attenuators (PA5) and a headphone driver (HB7). Acoustic stimuli were presented from an SEAS T 25 CF 002-06 loudspeaker placed 50 cm in front of the cage wall where the equipment for drinking water was placed. The frequency response varied less than \pm 4dB over the frequency range 2–8 kHz. As noted above, variability in the animal's head position during acoustic stimulation was minimized due to the design of the experiment: the stimulus was presented only when the animal was in contact with the spout. The sound intensity at the head position of the rat varied within 2 dB in the frequency range from 2 to 6 kHz, and within 4 dB in the range 6–8 kHz. The distortion product of the acoustic stimulation system was below –55 dB (0.18%) in the frequency range from 2 to 8 kHz.

Cortical lesion procedure and histological control

Unilateral or bilateral lesions of the AC were performed under deep anesthesia. Rats were anesthetized with a ketamine (Narkamon 5%, Spofa) and xylazine (Rompun 2%, Bayer) mixture (ratio 3 : 1, dose 0.1 mL/100 g b.w., i.p.). The depth of anesthesia was controlled by monitoring the tail flick reflex. The skin and underlying muscles were retracted in the area of the AC, and then the rats were placed in a stereotaxic apparatus. After identification of the bregma, the bone of the skull covering the temporal AC was removed between AP: -3.0 mm and AP: -7.8 mm. Laterally the opening exposed the rhinal fissure and medially reached up to the sagittal plane 6.0 mm. Lesioning the AC was performed by thermocoagulation. For thermocoagulation a custom-made apparatus was used with an electrically heated circular metallic plate (diameter of 4 mm) with adjustable temperature. The thermocoagulator was heated to ~ 150 °C and applied for ~ 1 s. The opening was packed with sterile Gelfoam and the incision was closed with a silk suture. Animals were treated with a local anesthetic (Mesocaine, Léčiva) and local application of a topical antibiotic powder (Framykoin, Infusia). The extent of the cortical lesion corresponded to the trepanation opening with the exception of its lateral margin, where it terminated 1-1.5 mm above the rhinal fissure. The essential criterion for including data obtained in an animal with a lesion into the final analysis was the fact that the lesion did not involve any part of the hippocampus. Sham operations were performed under the same conditions of anesthesia. In sham-operated animals, a partial trephine opening of the temporal bone on one or both sides was made. The bone was only raised and then replaced.

At the end of the experiments, a histological control of the location and extent of the lesion was performed. The rats were deeply anesthetized with pentobarbital (50 mg/kg i.p.) and perfused transcardially with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The animals were decapitated, the brains extracted from the skull and postfixed in the same fixative for 1-2 h (4 °C) and then cryoprotected with 30% sucrose in phosphate buffer overnight. Coronal sections of 40 µm thickness were cut with a freezing microtome. Every fourth section was stained with cresyl violet and mounted on slides. In order to analyse the three-dimensional extent of the cortical lesion, selected serial sections were redrawn and the boundaries of the cortical lesions transferred to standard sections of the Paxinos & Watson (1986) stereotaxic atlas. Delineation and nomenclature of the cortical areas was based on the atlas of Paxinos & Watson (1986) and the cytoarchitectonic criteria of Zilles (1985). Lesions in each animal were reconstructed by the alignment of serial coronal brain sections (Fig. 2). The care and use of animals reported on in this study were approved by the Ethics Committee of the Institute of Experimental Medicine and followed the guidelines of the Declaration of Helsinki.

Results

Discrimination training in control rats

Normal rats (n = 3) started to discriminate (χ^2 test, P < 0.05) between falling (6–3 kHz) and rising (3–6 kHz) FM tones in 2–6 training sessions and reached a steady level of DP in 8–11 training sessions. The duration of the discrimination training in sham-operated rats (n = 4) was similar to that in normal rats: they started to discriminate between stimuli in 2–4 sessions and reached a steady level of DP in 10–12 sessions. Further prolongation of the training period did not lead to any improvement in DP in either group. As an effect of training, the mean DP reached 61.9% in normal rats and 58.9% in sham-operated rats. The difference in the final DP after training



FIG. 2. Localization of lesions in the left (L) and right (R) hemispheres. Lesions made before the discrimination training: bilateral lesion of the AC in three rats (B1, B2 and B3), unilateral lesion of the left AC in three rats (L1, L2 and L3), unilateral lesion of the right AC in three rats (R1, R2 and R3). Lesions made after discrimination training: unilateral lesion of the left AC in three rats (TL1, TL2 and TL3), unilateral lesion of the right AC in three rats (TR1, TR2 and TR3). Areas of the AC indicated according to Zilles (1985): 1, Tel (temporal); 2, Te2; 3, Te3; 4, Par2 (parietal).

between normal and sham-operated rats was not significant (unpaired *t*-test, P > 0.05), and therefore normal and sham-operated rats were pooled together to create a single control group for subsequent comparison with lesioned rats. The mean value of the final DP for the control group was $60.2 \pm 4.2\%$ (Fig. 3). Differences in behavioral strategy were observed among control animals at the beginning of training. One of the strategies was to ignore the warning stimulus, which was reflected in low H and F values at the beginning of training (four rats). Improvement of the DP during training in these rats was achieved by increasing the hit rate. Another strategy was characterized by an avoidance response (breaking contact with the spout) to almost every presented stimulus (one rat). This strategy led to high H values as well as high F values. Improvement of the DP during training in this case was mainly the result of a decreasing false alarm rate. Other animals (two rats) at the beginning of training responded more or less randomly to both safe and warning stimuli, resulting in H and F values of about 50%; improvement of the DP in these rats was achieved by increasing the hit rate and decreasing the false alarm rate.

Discrimination training in rats with an AC lesion

Discrimination training was performed in three rats with a lesion of the left AC, in three rats with a lesion of the right AC and in three animals with bilateral lesions of the AC. The extent of the lesions in individual

animals is shown in Fig. 2. All rats with cortical lesions (both unilateral and bilateral) were able to learn to discriminate between falling and rising FM tones. However, the final DP after training in rats with AC lesions was significantly lower than in control animals: $51.2 \pm 5.6\%$ for left-lesioned rats (P < 0.05, unpaired *t*-test) and $40.5 \pm 6.2\%$ and $41.7 \pm 6.1\%$ for right- or bilaterally lesioned rats (P < 0.005, unpaired t-test), respectively (Fig. 3A). After training, the DP in rats with a left AC lesion was significantly higher than in rightor bilaterally lesioned rats (P < 0.005, unpaired *t*-test). The distribution of the DP values during the period of steady level performance, in the case of rats with a left AC lesion (Fig. 3B; top inset), overlapped with the distribution observed in control animals, showing that the best performances of rats with a left AC lesion are almost as good as the best DPs in control animals. By contrast, the distribution of DPs of rats with either a right or a bilateral AC lesion (Fig. 3C and D; top insets) barely overlapped with the distribution seen in control animals, indicating that the best performances of rats with either a right or a bilateral AC lesion only infrequently reached the level of the worst DPs in control animals.

Left lesioned rats started to discriminate between rising and falling FM tones in 2–4 days (χ^2 test, P < 0.05) and reached a steady DP level after 18 or more days of training. Two right-lesioned rats (R1, R3) achieved a relatively high DP (around 40%) in the first 3 days of training (Fig. 3C). The third rat (R2) began to discriminate between the FM tones on the 4th day of training (χ^2 test, P < 0.05) and reached a steady level on the 9th training day. Subsequent training did not lead to any improvement in the DP in these rats; only fluctuations around the attained steady DP level were observed. Rats with bilateral lesions of the AC started to discriminate between rising and falling FM tones within the first 3 days of training (χ^2 test, P < 0.05) and, similarly to the right-lesioned rats, achieved relatively high (26-40%) DP values in the first days of the training (Fig. 3D). However, their DP was very unstable and demonstrated considerable variability, i.e. relatively high DP values alternated with very low values. DP reached a steady level after 17-21 days of training. The variability in the extent of the lesion and its position in the cerebral cortex did not significantly influence the final DP value (see Supplementary material, Fig. S3).

In animals trained after a unilateral or bilateral lesion of the AC, the strategy of ignoring the warning stimulus was observed in two leftlesioned rats and two right-lesioned rats; the strategy of random responses to both safe and warning stimuli was found in one leftlesioned rat and in one bilaterally lesioned rat; the strategy of avoiding every presented stimulus was observed in one right-lesioned rat and two bilaterally lesioned rats.

Testing the ability to discriminate novel pairs of stimuli in control rats and in rats trained after an AC lesion

After ending the training to discriminate between a rising 3–6 kHz FM tone and a falling 6–3 kHz FM tone, the spontaneous transfer of the conditioned response to novel pairs of stimuli was tested in control rats and in rats with an AC lesion.

When the rats, instead of discriminating between rising and falling FM tones, had to discriminate between two pure tones (novel pair I: a 6-kHz tone was used as a warning stimulus and a 3-kHz tone as a safe stimulus, Fig. 1B), they demonstrated high DP values in the first session. These values reached 58.5 and 56.5% for rats of the control and left-lesioned groups, respectively (Fig. 4), and did not differ significantly (paired *t*-test) from the final DP attained in discrimination training with rising and falling FM tones. For the right- and bilaterally lesioned rats, DP in the pure tone discrimination test was significantly



FIG. 3. Results of discrimination training in rats with AC lesions. (A) Final discrimination performance in control and lesioned rats, mean \pm SD (*P < 0.05; **P < 0.005, unpaired *t*-test). (B–D) Time course of DP in left-lesioned rats, right-lesioned rats and bilaterally lesioned rats, respectively (individual data); the grey area indicates the DP range observed in the control group (mean \pm SD, smoothed curve); top insets – histograms of distribution of DPs during the period of steady level performance for the control (grey) and lesioned (open) groups. The arrows indicate the final DPs of individual groups.



FIG. 4. Comparison of the discrimination performance in response to novel tonal stimuli (first session) with the results of the initial discrimination training. Discrimination performance in response to novel tone pair I (a warning pure tone at 6 kHz vs. a safe pure tone at 3 kHz) – left column – and for novel tone pair II (a warning pure tone at 3 kHz vs. a safe pure tone at 6 kHz) – right column. Grey area indicates the final discrimination performance (mean \pm SD) in response to the initial training stimuli (warning, falling from 6 to 3 kHz; safe, rising from 3 to 6 kHz).

better (P < 0.005, paired *t*-test) than the final DP obtained in the initial discrimination training with FM tones (61.2 vs. 40.5% and 75.1 vs. 41.7%, respectively; Fig. 4).

When the meaning of the same pair of pure tones was reversed (novel pair II: the 3-kHz tone was used as the warning stimulus and the 6-kHz tone as the safe stimulus, Fig. 1C), the DP values were negative in all groups: -17.8, -1.2, -14.1 and -24.1% for control, left-, right- or bilaterally lesioned groups, respectively (Fig. 4). The negative DP values mean that the rats stopped drinking when the safe stimulus was presented and continued to drink throughout the warning stimulus presentation. These findings indicate that information about the frequency at the beginning of the stimulus could serve as an important cue for the discrimination between FM stimuli both in control and lesioned rats, especially in rats with a destruction of the right AC.

On the basis of these findings, we assumed that the increase in the modulation rate of rising and falling FM stimuli could reduce the discrimination ability of the rats and that using FM stimuli that start their rise and fall from the same frequency would make the discrimination task still more difficult. Therefore, the animals were exposed to a third novel pair: the FM stimuli were in the same frequency range (falling 6–3 kHz and rising 3–6 kHz) but with a higher frequency modulation rate (4 oct./s in comparison with the

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initial 1.3 oct./s, Fig. 1D). All rats with the exception of one bilaterally lesioned rat were able to discriminate between these novel stimuli with faster FM (χ^2 test, P < 0.05) already in the first session; however, the DP achieved in the first test session was significantly worse (P < 0.005) than the final DP attained in the discrimination training (Fig. 5). One week's training in discriminating between these stimuli resulted in a DP improvement in all groups (P < 0.05, paired t-test). The mean group DP values in control and left-lesioned rats after 1 week of training reached 47.2 and 41.0%, respectively. By contrast, the mean DPs in the right and bilaterally lesioned rats amounted only to 26.6 and 27.4%, respectively, and were significantly lower than in control animals (P < 0.005, unpaired *t*-test) and in the left-lesioned rats (P < 0.05, unpaired *t*-test). When the rats were tested to discriminate between FM tones starting from the same frequency (novel pair IV: a falling 4-2 kHz FM tone was used as a warning stimulus and a rising 4-8 kHz FM tone was used as a safe stimulus, Fig. 1E), the mean DP value in the first test session was near zero in all groups and in the majority of animals was slightly negative. One week of training resulted in a significant DP improvement (P < 0.005, paired t-test) in control and left-lesioned rats; DP in these groups reached values of 50.2 and 37.7%, respectively (Fig. 6). No animal with a right AC lesion or with a bilateral lesion was able to discriminate significantly (χ^2 test, P > 0.05) between rising and falling stimuli starting from the same frequency even after 1 week of training.

Effects of AC lesions on discrimination between FM tones in trained rats

In six rats, a lesion of the right or left AC was made after discrimination training (between FM tones falling from 6 to 3 kHz and rising from 3 to 6 kHz). The final DP attained by these rats before lesioning was $61.3 \pm 4.9\%$. Testing conducted after 7 days of recovery demonstrated that the DP values declined to $13.4 \pm 6.2\%$ in the rats with a lesion of the left AC (n = 3) and to $6.2 \pm 2.9\%$ in rats with a lesion of the right AC (n = 3) (Fig. 7). Only one rat with a left AC lesion was able to discriminate between rising and falling FM stimuli (χ^2 test, P < 0.05) in the first test sessions after lesioning. The worsening of the DP after lesioning was mainly due to a marked increase in the false alarm rate. All trained rats, with the exception of the aforementioned left-lesioned rat, used in the first postoperative session a strategy of avoiding every presented FM stimulus, which resulted in high values of both hit rate and false alarm rate.



FIG. 5. Comparison of the discrimination performance using FM tones with a faster modulation rate than the original FM tones with the results of the initial training. Discrimination performance in response to novel FM tones (warning, falling from 6 to 3 kHz; safe, rising from 3 to 6 kHz, with a modulation rate of 4 oct./s) in the 1st session (left column) and in the 7th session (right column). Grey area indicates the final discrimination performance (mean \pm SD) in response to the initial training stimuli (warning, falling from 6 to 3 kHz; safe, rising from 3 to 6 kHz). **P* < 0.05 and **P* < 0.005, unpaired *t*-test.



FIG. 6. Comparison of the discrimination performance in response to novel FM tones rising and falling from the same frequency with the results of the initial discrimination training. Discrimination performance in response to novel FM tones rising and falling from the same frequency (warning, falling from 4 to 2 kHz; safe, rising from 4 to 8 kHz) in the 1st session (left column) and in the 7th session (right column). Grey area indicates the final discrimination performance (mean \pm SD) in response to the initial training stimuli (warning, falling from 6 to 3 kHz; safe, rising from 3 to 6 kHz). *P < 0.05; **P < 0.05, unpaired *t*-test.



FIG. 7. Time course of the discrimination performance (mean \pm SD) during postlesion training in rats with a left (filled diamonds) or right (filled squares) lesion of the AC made after the initial training (discrimination between warning, falling 6–3 kHz and safe, rising 3–6 kHz FM tones). Grey area indicates the final discrimination performance (mean \pm SD) in response to the initial training. Unfilled diamonds and unfilled squares – DP in response to pure tones (warning 6 kHz and safe 3 kHz) in rats with a left or right lesion of the auditory cortex.

When instead of FM tones a novel pair of pure tones (a 6-kHz tone as a warning stimulus and a 3-kHz tone as a safe stimulus, Fig. 1B) was presented in the second or third postoperative session, all rats demonstrated a high DP, 53 ± 4 and $55 \pm 5\%$ for left- and rightlesioned rats, respectively (Fig. 7). Three weeks of retraining with FM stimuli resulted in a considerable improvement in the DP in rats with a left AC lesion but only a slight DP improvement in rats with a right AC lesion. DP in the left-lesioned rats increased to $48.1 \pm 3.5\%$, thus approaching the final DP attained in the discrimination training before lesioning, but was still significantly lower (P < 0.05, paired *t*-test). In the right-lesioned rats, the DP value after retraining was only $20 \pm 5\%$. The DP increase in the retraining period was mainly connected with a decreasing number of false alarm reactions. In two rats with a right AC lesion, the decrease in the false alarm rate during retraining was practically absent. The ability of animals with cortical lesions to discriminate pure tones was similar at the end of the retraining period to that seen at the beginning (Fig. 7).

Discussion

The results of our study demonstrate that rats with a unilateral or bilateral ablation of the AC were able to learn to discriminate between falling and rising FM tones; however, they displayed worse DP than did normal control rats. A greater deficit in the ability to discriminate between rising and falling FM tones was observed in animals with a lesion of the right AC and animals with bilateral lesions; the final DP in these rats was significantly worse than the DP in rats with a left AC lesion.

The individual variability of the final DP in rats within the same experimental group was relatively small, indicating their similar discrimination ability (Supplementary material, Figs S1A and S3). The differences in behavioral strategies, which were apparent in the values of the hit rate and false alarm rate at the beginning of training (Supplementary material, Fig. S2) in both control and lesioned rats, had no effect on the final result of the training (Supplementary material, Fig. S1). The hit and false alarm rates, which fully describe performance in a discrimination YES/NO task, reflect two factors: sensory (the perceptual origin of the animal's behavior) and response bias (the general tendency to respond YES or NO, as determined by the response criterion). The response criterion may be more liberal (lax), which biases the subject towards responding YES regardless of the stimulus, resulting in a high hit rate and a high false alarm rate, or more conservative (strict), which biases the subject towards responding NO, resulting in a low false alarm rate but also a low hit rate (Green & Swets, 1966; Stanislaw & Todorov, 1999). In our experiments, the strategy characterized by an avoidance response (breaking contact with the spout) to almost every presented stimulus reflected a liberal criterion, and the strategy characterized by drinking in the presence of the warning stimulus reflected a conservative criterion. During training the values of the hit rate and false alarm rate converged to similar values, independent of the initial strategy (Supplementary material, Fig. S1, B and C).

Our findings indicate that the right AC plays a leading role in discriminating the direction of a sound frequency change; rats with a right or bilateral AC lesion improved their DP mainly by recognizing the pitch at the beginning of the FM stimuli. Cerebral asymmetries are well known in humans. The functional dominance of the left hemisphere for verbal stimuli has been demonstrated in many studies and through clinical observation of patients with surgical lesions (e.g. Hellige, 1990; Fitch et al., 1997). Concurrently, other studies revealed the right hemisphere preference in perceiving the pitch of complex tones and pitch or timbre discrimination (Sidtis, 1980; Zatorre, 1988; Van Lancker, 1997; Johnsrude et al., 2000). Recent neuroimaging and neuropsychological data suggest that FM directional discrimination is associated with significantly greater activity in the right lateralposterior superior temporal sulcus and lateral middle temporal gyrus (Poeppel et al., 2004). Most data concerning hemispheric asymmetry in animals point to left hemisphere specialization for the processing of species-specific vocalizations and temporal acoustic information, a feature that could represent an evolutionary precursor to lateralized speech perception and language processing in humans (Heffner & Heffner, 1984; Petersen et al., 1984 - in monkey; Nottebohm, 1980; Williams, 1990 - in different species of passerine birds; Ehret, 1987; Geissler & Ehret, 2004 - in mice). The specialization of the right hemisphere was revealed in experimental animals by Wetzel et al. (1998), who demonstrated that gerbils with a right AC lesion were

impaired in FM directional discrimination learning, whereas gerbils with left AC lesion were indistinguishable from controls. Poremba et al. (2004) described two types of hemispheric lateralization in the monkey AC: left hemisphere specialization for the processing of monkey calls and right hemisphere specialization for the processing of a wide variety of acoustic stimulus classes. Our findings, indicating an important role for the right AC in the processing of FM stimuli, support data by Wetzel et al. (1998) and correspond to the results of Poremba et al. (2004). In contrast to the results of Wetzel et al. (1998), who found that gerbils with a right AC lesion were not able to learn to discriminate between rising and falling FM tones, our data indicate that rats with a right AC lesion are able to accomplish this task, although they have noticeably worse DP than control rats or rats with a lesion of the left AC. Evidently, there are several reasons why the outcomes of our experiments and those of Wetzel et al. (1998) are different: there are differences in the avoidance method used, in the stimuli parameters (different modulation rates) and in animal species (rat vs. gerbil). It should also be noted that the results of Wetzel et al. (1998) concern only the first 8 days of training, whereas we investigated the learning of the rats for a longer period. In another study, these authors (Ohl et al., 1999) described an impaired ability to discriminate FM tones after a bilateral lesion of the AC; in this case, the DP of gerbils improved over the course of 14 training days. According to Kelly & Whitfield (1971), cats with bilateral lesions of the AC have 'difficulty in learning'; however, they demonstrate a capacity to discriminate between rising and falling tones.

On the basis of the results of the present study and literature data indicating that sound frequency discrimination can be successfully learned by animals with bilateral lesions of the AC (Elliott & Trahiotis, 1972; Neff et al., 1975; Ohl et al., 1999), we hypothesize that rats with a lesion of the right AC can learn to discriminate between rising and falling FM tones without discerning the direction of frequency modulation, but only by identifying the frequency at the beginning of the stimulus. Our findings indicate an important role for information about the frequency at the beginning of a stimulus in the discrimination of FM tones for all groups of rats in our experiment, especially for rats with a lesion of the right AC. When the rats, trained to recognize FM tones, had to discriminate between two pure tones that were of the same frequency as the frequency at the beginning of the FM tones, all rats demonstrated high DP values in the first session. For rats with right or bilateral cortical lesions, the discrimination between pure tones in the first session was easier than the discrimination between FM tones after 3 weeks' training. By contrast, the presentation of the same pair of pure tones with a reversed signal meaning elicited a paradoxical response (stopping drinking in response to the safe stimulus and continuing drinking in response to the warning stimulus). The introduction of novel stimuli with an FM faster than that used in the initial training complicated the discrimination task. In this experimental situation, right-lesioned rats and bilaterally lesioned rats showed the greatest deficit in their ability to discriminate novel FM tones, both in the first session and after 1 week's training. Finally, the discrimination of novel FM stimuli when both the increasing and the decreasing tone frequencies started from the same tone appeared to be the most difficult task for both control and lesioned rats. In this case, the first test session DPs were near zero or even slightly negative. The significant increase in DP in only control and left-lesioned rats, and the lack of improved DP in right-lesioned and bilaterally lesioned rats, after 1 week of training with these stimuli indicates again that the right AC plays an important role in the discrimination of the direction of frequency modulation. It should be noted that retraining difficulties could be

aggravated because in this novel FM pair, the frequency ranges were different from those used in the initial FM pair.

Evidently, rats with an AC lesion (especially on the right side) may have specific problems with analysing frequency variations over time. In this respect, our data support the idea that the AC is essential for discrimination in tasks that require the identification of the temporal structure of acoustic stimuli (discrimination of the temporal pattern of a tone chain – Diamond & Neff, 1957; Kelly, 1973; discrimination of the gap in noise – Ison *et al.*, 1991; Kelly *et al.*, 1996; Syka *et al.*, 2002).

The study of DP after a lesion of the AC in rats that were previously trained to discriminate between rising and falling FM tones allows the acquisition of additional data supporting the importance of the AC for this kind of auditory discrimination. Lesions of both the right and the left AC in rats led to a considerable worsening of the DP. Worsening of the DP was mainly due to a marked increase in false alarm reactions. Increased numbers of false alarm reactions were also described after bilateral lesions of the AC in cats by Kelly & Whitfield (1971) and in gerbils by Ohl et al. (1999). According to Kelly & Whitfield (1971), a high rate of false alarm reactions results directly from a cortical lesion and may hinder the animal in performing discrimination tasks or, far more likely, reflects the difficulty the animal has in making such discriminations. Ohl et al. (1999) hypothesized a cortical role in inhibiting the reaction to nonreinforced stimuli in conditioning experiments and considered that arousal may play a significant role in the observed failure to inhibit false alarm reactions after a cortical lesion in trained rats. It seems that trained rats barely identify warning stimuli after lesioning and try to avoid a shock by using the strategy of avoiding every presented stimulus. This leads to increasing false alarm responses combined with a relatively high percentage of correct responses, finally resulting in a decrease of DP. The high DP values observed in the first postoperative sessions in the discrimination of novel pure tones indicate that the deficit in the discrimination between FM tones reflects an impaired ability to recognize the stimuli rather than a broader generalization across stimuli, leading to an avoidance reaction to both warning and safe stimuli. The spontaneous transfer of the conditioned response to novel tone stimuli observed after lesioning in trained rats indicates an ability to retain the conditional avoidance response that was formed before lesioning. This fact suggests that the impaired discrimination between FM tones is connected with impaired sensory processing rather than with possible emotional, motivational or motor disturbances caused by the AC lesion.

Our findings indicate that an intact AC is necessary for discrimination of the FM sounds and that the right hemisphere plays a dominant role in the recognition of the direction of frequency modulation. This study is the first to report different roles for the left and right auditory cortices in the processing of complex auditory stimuli in the rat, a species frequently used in auditory neuroscience.

Supplementary material

The following material is available online at http://www.blackwell-synergy.com

Fig. S1. Different behavioral strategies and parameters of rats' performance (DP, hit rate, false alarm rate) during discrimination training.

Fig. S2. Reflection of different strategies in the hit rate and false alarm rate.

Fig. S3. The relationship between the extent of the lesion and the final discrimination performance of individual animals.

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Abbreviations

AC, auditory cortex; DP, discrimination performance; F, false alarm rate; FM, frequency modulation; H, hit rate.

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