

Generalization of conditioned suppression during salicylate-induced phantom auditory perception in rats

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Abstract. Tonal frequency generalization was examined in a total of 114 pigmented male rats, 60 of which were tested under the influence of salicylate-induced phantom auditory perception, introduced before or after lick suppression training. Thirty control subjects received saline injections, and the remaining 24 subjects served as noninjected controls of tonal background effects on generalization. Rats were continuously exposed to background noise alone or with a superimposed tone. Offset of background noise alone (Experiment I), or combined with onset or continuation of the tone (Experiments II and III) served as the conditioned stimulus (CS). In Experiment I, tone presentations were introduced only after suppression training. Depending on the time of salicylate introduction, a strong and differential influence on generalization gradients was observed, which is consistent with subjects' detection of salicylate-induced, high-pitched sound. Moreover, when either 12- or 3 kHz tones were introduced before or after Pavlovian training to mimic salicylate effects in 24 rats, the distortions in generalization gradients resembled trends obtained from respective salicylate injected groups. Experiments II and III were aimed at evaluating the masking effect of salicylate-induced phantom auditory perception on external sounds, with a 5- or a 10-kHz tone imposed continuously on the noise or

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presented only during the CS. Tests of tonal generalization to frequencies ranging from 4- to 11- kHz showed that in this experimental context salicylate-induced perception did not interfere with the dominant influence of external tones, a result that further strengthens the conclusion of Experiment I.

Key words: auditory, frequency, generalization, hallucinations, licking, rats, perception, salicylate, suppression

INTRODUCTION

Previously, we have reported a model that objectively assesses subjective auditory phantom perception in rats through their observable behaviour (Jastreboff et al. 1988a). These experiments offer a behavioral manifestation of auditory phantom perception, reported in humans as tinnitus, and are supported by a variety of physiological and electrophysiological data as well (Jastreboff and Sasaki 1986, Jastreboff et al. 1987, 1988b, 1988d). Tinnitus is a widespread disorder of the human auditory system, affecting 17% of young and adult populations and up to 30% of the elderly (Coles 1987, Salomon 1989), without any established method of alleviation. The model is crucial to determine the mechanisms of this disorder under proper experimental scrutiny, not possible by investigation on humans only.

Within a conditioned suppression paradigm, subjects were given daily injections of salicylate, a substance known to cause auditory phantom sensations systematically in humans (Flower et al. 1980, McFadden 1982, Mongan et al. 1973). In an electrophysiological study of tinnitus, the spontaneous activity in cells of the inferior colliculus increased after salicylate administration, while spontaneous activity in the nonauditory part of cerebellar vermis remained stable (Jastreboff and Sasaki 1986). These findings supported the hypothesis that tinnitus results from an aberrant neural activity within the auditory pathways. Presumably, this activity is erroneously interpreted as sound at higher auditory centres.

In the Jastreboff et al. (1988a) experiments, contrasting results were obtained in lick suppression levels tested in extinction, dependent on the sequence of

initiating salicylate injections in relation to acquisition training sessions – i.e., before or after Pavlovian suppression training. Relative to control subjects injected with saline, the overall pattern of behaviour in subjects starting injections prior to training was marked by more severe suppression and persistence of suppression. Conversely, extinction response levels of subjects beginning injections after training showed characteristically less suppression and rapid recovery of drinking. This pattern was reliable across changes in thirst motivation.

However, those group differences were eliminated when the sensory modality was switched to visual, indicating that the salicylate-induced effect was specifically auditory. Furthermore, we obtained analogous results using another drug, quinine (Jastreboff et al. 1988c) which presumably has a different mechanism of action on the auditory system than salicylate (Puel et al. 1990, Smith et al. 1985) but shares with salicylate, tinnitus inducing properties in humans (Flower et al. 1980, McFadden 1982). Detailed analyses and interpretations of these results are presented in previous papers (Jastreboff et al. 1987, 1988a, 1988b, 1988c), which collectively support the conclusion that this paradigm affords the detection of salicylate-induced phantom auditory perception in rats.

While reports of salicylate-induced tinnitus in humans typically indicate high pitch sound, other types of tinnitus exhibit wide variability (Coles 1987, Leske 1981, McFadden 1982), ranging from discrete tones to complex sounds at a variety of loudness levels. We wanted to determine whether this behavioural model of tinnitus is able to assess the psychoacoustical parameters of subjective auditory perception and estimate loudness, pitch, and the masking properties of tinnitus. Results with different doses of salicylate or

quinine show that the behaviour of rats progressively depends on the dosage of salicylate administered (Jastreboff et al. 1988b, 1988c). Since human data indicate a relationship between drug dosage and the loudness of tinnitus, it seemed reasonable to postulate that the suppression paradigm may be sensitive to the perceived loudness of tinnitus. The important question of assessing the pitch of induced auditory phantom perception remained open. Accordingly, three related experiments investigated the possibility of evaluating the perceived pitch of tinnitus and the extent to which tinnitus may interfere with the perception of external auditory signals.

EXPERIMENT I. GENERALIZATION OF SALICYLATE-INDUCED PERCEPTION

The first experiment was directed at the evaluation of the perceived pitch of salicylate-induced phantom auditory experience. Human reports characterize salicylate-induced tinnitus as a high pitch tone or narrow band noise, which can be matched to the pitch of external tones (McFadden and Plattsmier 1983, McFadden et al. 1984). This observation suggests the possibility of using tinnitus induced just prior to conditioned suppression training as a CS and to evaluate its frequency generalization during extinction of suppression behaviour. It was expected that tinnitus pitch may generalize to novel frequency values presented during extinction.

Method

SUBJECTS

The method followed the paradigm reported previously for detection of tinnitus in rats (Jastreboff et al. 1988a). Forty-eight pigmented rats, descendent from Charles River strains and bred in the animal colony of the University of Massachusetts at Boston were used for this experiment. At the time of water deprivation, subjects' weights ranged from 377 g to 413 g. They were housed singly with food always available. For the duration of the experiment, individual cages were stored in a single sided 1.5 x 2 m rack, which could accommodate five rows of three cages and was kept in a 3.5 x 4.5 m cubicle, separate from the main colony. Animals were water deprived and were weighed daily to ensure that their weight loss did not exceed 20% of their

pre-deprivation levels. None of the subjects required water outside of experimental sessions to keep their weight at 80% ad libitum levels. Subjects were maintained on a 12 h light/dark (7:00 am/7:00 pm) cycle throughout the experiment. Daily sessions began at 8:00 am, and subjects were run in squads of four rats in the same ordered sequence.

APPARATUS

Acquisition training and extinction testing were conducted in four plexiglass rodent cages (BRS/LVE RTCO24), with interior dimensions measuring 26- x 29.5- x 23.25-cm. The four cages used in this experiment were yoked for presentation of all experimental events. Each cage was housed in an acoustically shielded environmental cubicle (BRS/LVE Sec-002). The floor of each cage consisted of 1.5 cm steel shock grids spaced 1.4 cm apart. Pulsed shock of 1.5 mA was delivered from a matched impedance shock source through a scrambler (BRS/LVE SGS-004). The only illumination within each cage was provided by a weak house light centred at the top of the wall containing the drinking tube. Drinking tubes identical to those available in the home cages were connected to a circuit consisting of the tube itself and the floor of the cage, so that contact during a licking response was recorded by a drinkometer (Lafayette, Model 58008). Two Soundalert speakers (Model SC628), 3 cm in diameter, were mounted on the wall 15 cm and 18.5 cm above the drinking tube. A 9 cm wide Motorola speaker (P35-VAH) was placed on a table located 1 m in front of the centre of the animal storage rack. Background noise presentations in the experimental cages and in the colony room were provided by Hewlett Packard Precision Noise Generators (Model 8057A) and were exactly the same as those used previously in the basic experiments on auditory phantom perception (Jastreboff et al. 1988a). The sound pressure levels (SPL) close to the drinking tubes in the experimental chambers as well as in the home cage storage room were measured by a Precision Sound Level Meter (Quest Electronics, Model 155) in 1/3 octave bands (Quest Electronics 1/3 Octave Filter, Model OB-133) and were presented previously (Jastreboff et al. 1988a). The SPL level was calculated for frequencies of 1-16 kHz, according to standard procedure (Durrant and Lovrinic 1984), to be 62 dB SPL. Pure tones of 4 to 11 kHz in 1 kHz intervals were produced by a Wavetek Function Generator, Model 182A. The sound pressure at the centre of the licking chamber was adjusted to be

65 dB SPL for all the tones used. Each speaker was calibrated individually to insure that all the levels were within a decibel of each other.

Presentation of experimental events and all recording were accomplished by a program governing a Zenith 159 computer with interface and related software, Quick I/O™. Individual session records were stored on floppy disks as well as on printouts using an Epson RX-80 Printer.

PROCEDURE

All subjects followed the same sequential pattern consisting of initial lick training, Pavlovian acquisition of conditioned suppression, and extinction testing as described earlier (Jastreboff et al. 1988a). Prior to lick training, subjects were weighed and deprived of water. Weight loss to 80% of initial levels typically required three days to reach. Lick training began 24 h later and continued for at least two days, until a minimum of 350 licking responses were recorded during a 45 min session. The typical number of licks per session was in the 1,000–2,000 range. During each subsequent training and testing session, the cumulative numbers of licks were recorded for each subject every 30 s. When fully trained, subjects in this experiment averaged 12.5 ml per session (range: 6–18.5 ml), which was entirely consistent with the daily volume of drinking obtained in the Jastreboff et al. (1988a) experiments. Beginning with the first day of lick training, subjects were exposed continuously in the home cage and in the experimental chamber to the 62 dB SPL wide band background noise, the spectrum of which was presented in Fig. 1 of the Jastreboff et al. (1988a) paper.

On the day following lick training, all subjects received an acclimation session during which the to-be-conditioned stimulus, consisting of a 1 min period of offset of the noise, was presented five times within the session at random intervals, centred around times approximately 3–, 10–, 21–, 28–, and 34 min after the start of the session. The exact time of each CS probe was varied randomly across subsequent experimental days. All subjects received two days of suppression training, during which each of the five CS probes terminated with a 0.5 s pulse of 1.5 mA footshock. Subjects were assigned to one of three groups:

Salicylate before (SB). Eight rats were given daily s. c. injections of 350 mg/kg sodium salicylate (corresponding to 300 mg/kg salicylate acid), 2 h before the first

Pavlovian suppression training session. On each succeeding experimental day, subjects receive a salicylate injection 2 h before each session.

Salicylate after (SA). Eight subjects were treated in the same manner as the SB group, except that the salicylate injections began at the conclusion of the second Pavlovian training session and continued 2 h prior to each remaining session.

Control (C). Eight rats received daily injections of saline following the acclimation session and 2 h before each subsequent session.

In addition, four other groups of six rats were used to test whether the introduction of specific tones in the same relationship to training as the salicylate injections – i.e., before or after training – would serve a functionally equivalent effect as the drug. Accordingly, four groups were defined as follows:

12 kHz before (12B). Six rats were exposed continuously to a discrete tone of 12 kHz, 65 dB SPL, introduced 2 h before the first training session in the home cage and remained on both in the experimental chamber as well as in the home cage for the remainder of the experiment.

12 kHz after (12A). This group of six rats received the continuous 12 kHz, 65 dB SPL tone beginning 2 h before the first extinction sessions, and it remained on for the remainder of the experiment.

3 kHz before (3B). This group ($n=6$) received the same treatment as the 12B group, except that the tone imposed beginning prior to the first training sessions was at a frequency value of 3 kHz, 65 dB SPL.

3 kHz after (3A). The remaining six rats were exposed to 3 kHz beginning 2 h prior to the first extinction session.

Following suppression training, tonal frequency generalization testing proceeded during 5 days of extinction. On each test session, five novel tones of 7–, 8–, 9–, 10– and 11– kHz (all at 65 dB SPL) were presented once during the 1 min interval of noise offset. The order of generalization test stimuli was counterbalanced across subjects and extinction test session.

Since a wide range of individual differences in the numbers of licks occurred in subjects of this study as well as in others using similar procedures (e.g., Baum and Jacobs 1989, Boyd 1986, Matzel et al. 1988), a measure of behavioural change is used which references changes in licking to the level of ongoing behaviour. The "Kamin Ratio" of behavioural suppression measures the number of licks during tone offset, CS periods

(B) compared to the number of licks in the immediately preceding 1 min interval (A) through the ratio: $R_B = B/(A + B)$ (Annau and Kamin 1961, Church 1969). Accordingly, a ratio close to 0.5 indicates no suppression to the CS, while a ratio near 0.0 reflects almost complete CS suppression. To meet the criterion on the acclimation day, the mean lick ratios of individual subjects were expected to fall between 0.40 and 0.60, indicating no CS effect, which all subjects met. The criterion for acquisition of conditioned suppression was defined as individual ratios of 0.20 or less, which all subjects met within the two Pavlovian training sessions.

In each daily experimental session, five ratios of lick suppression were collected from each subject. These ratios were analysed by mixed design ANOVAs for partially correlated data, followed by Duncan Tests (Liquist 1953, Ridgman 1975) all of which were done with SASTM software.

Results

The results of Experiment I yielded clear effects from salicylate treatment. Markedly different generalization curves were obtained from each of the two salicylate injected groups and the saline control group, which are presented in Fig. 1.

Although the data were analysed utilizing all independent variables (groups, frequencies, days, repetitions), the major significant findings in this and subsequent figures are presented using means

for a given group and frequency, with data averaged over the remaining dimensions.

The saline control subjects (circles) showed some general suppressed levels to all tested frequencies, with an indication for some frequency gradient present ($F(4/35) = 2.66, p < 0.05$). The SA group (Fig. 1, triangles) showed a flat, slightly elevated gradient, which was confirmed by the lack of any significant effect from tonal frequency ($F < 1$). In contrast, the SB group (Fig. 1, squares) showed little response suppression at the lower frequency values, but responding was almost completely suppressed at the two higher frequency values, producing a very sharp gradient ($F(4/35) = 18.50, p < 0.0001$). Duncan tests of the frequency effect in the SB group indicated that responding to the 7-kHz values differed from response levels to all other values, while the suppression to the 8- and 9-kHz values were different from all others but not each other, and a similar relation was found between the 10- and 11-kHz values (all $ps < 0.05$). This trend in differential responding was consistently found in all subjects and continued throughout extinction sessions.

The statistical analysis of the mean ratios, R_B , of suppression during the tones for all groups on all sessions, supported the impression from Fig. 1. The highly significant main effect of group differences ($F(2/21) = 17.77, p < 0.0001$), emanating from the different sequence of salicylate injection relative to Pavlovian training in the SB and SA groups compared to the controls, was striking, yet group effects were not exactly ordered according to expectations from our

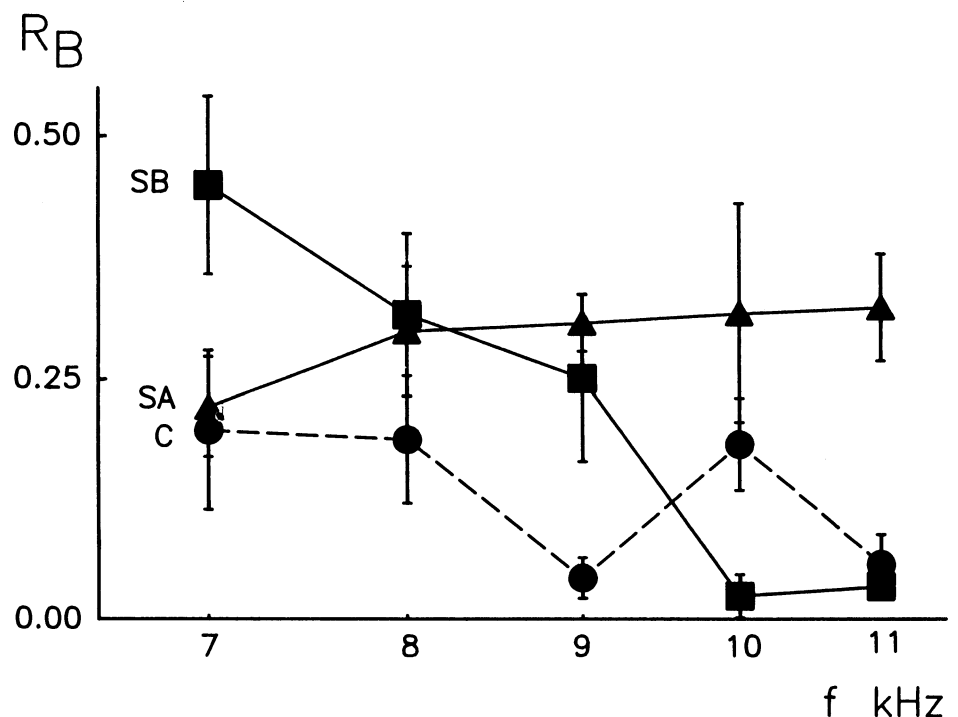


Fig. 1. Mean response ratios and related SEMs for control group (C, circles and dashed lines) and groups starting salicylate injection before (SB, squares) or after (SA, triangles) training and introduced to the external tonal stimuli on the 1st extinction session in Experiment I. Each mean represents 8 subjects given 5 testing probes on each of 5 successive extinction days.

earlier study when suppression was tested without additional tonal stimuli in extinction. While the SA group did show less suppression overall than the other two groups, performance in all of the groups, especially the SB group was affected by the addition of novel tones, relative to the reference level without tones. All groups within their respective response patterns showed less suppression with increasing generalization testing sessions, which was supported by a main effect of progressive Sessions ($F(4/84)=12.72$, $p<0.0001$) and the lack of a Group \times Sessions interaction ($F<1$). This nondifferential extinction effect was expected on the basis of the literature reporting generalization gradients tested in extinction (Brennan and Riccio 1973, Guttman and Kalish 1956, Hoffaman 1969).

The influence of novel tonal stimuli during CS probes was different in each group. In addition to the overall main effect of tonal Frequency variation ($F(4/84)=4.15$, $p<0.005$), the interactions of Group \times Frequency ($F(8/84)=5.16$, $p<0.0001$) and Sessions \times Frequency ($F(16/336)=1.68$, $p<0.05$) attained significance, while the remaining interaction of Group \times Sessions \times Frequency was marginally significant ($F(32/336)=1.47$, $p<0.0519$).

Of particular importance is the highly significant Group \times Frequency interaction indicating dissimilar processes of generalization in the treatment groups. Analysis of these gradients as evaluated by Duncan tests confirmed the impression from Fig. 1 of contrasting gradients in the SA and SB groups. The results of these tests are summarized in Table I, which suggests

that group differences in suppression tended to be more pronounced at the higher frequencies.

Figure 2 depicts the mean ratios over the seven extinction sessions to each of the novel generalization values for the two tonal control groups given the additional 12 kHz tone, while Fig. 3 indicates the same measure for the 3 kHz groups. In each figure the average suppression ratios to the test stimuli for the groups beginning additional tones before suppression training (12B and 3B) are indicated by the squares, while the triangles indicates mean gradients of sup-

TABLE I

Significant Duncan comparisons (all $ps<0.05$) of group differences in suppression to the generalization test stimuli of Experiment I, upper right. The lower left half of the matrix indicates which groups had less suppression in each of the respective matches pairs

		C	SA	SB
C		—	9 10 11	7 9
	SA	SA	—	7 10
	SA	SA		
SB	SB	SB	SB	11
	SB	SB	SA	—
	SB	SB	SA	

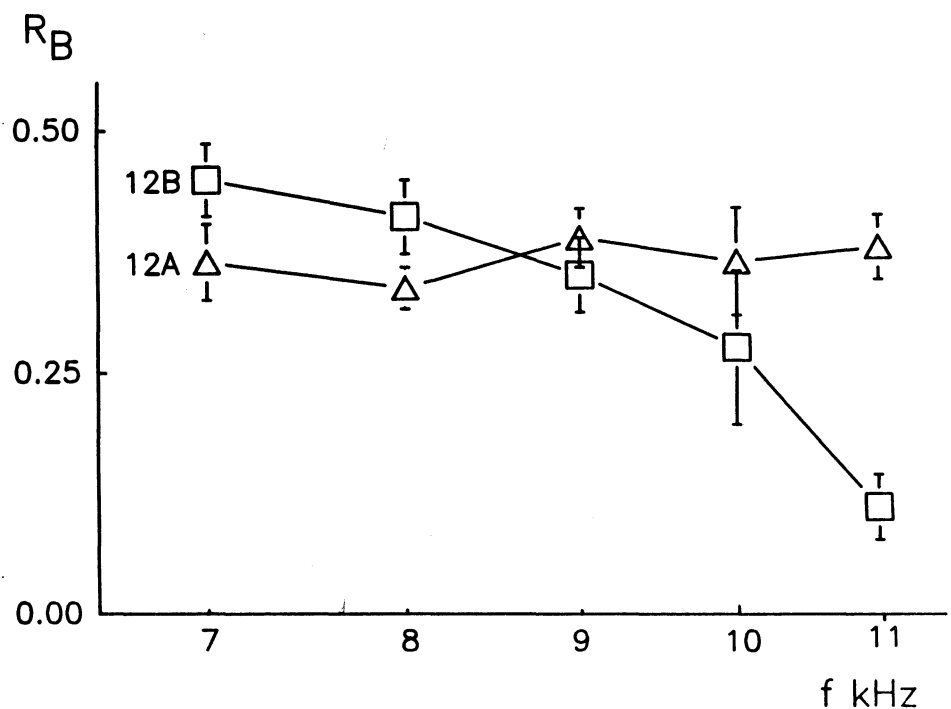


Fig. 2. Mean suppression during generalization to novel stimuli of control groups exposed continuously to 12 kHz beginning either before Pavlovian suppression training (12B, open squares) or after training (12A, open triangles).

pression for the groups starting additional tones after training. A cursory comparison of both figures indicates two effects. First, introduction of the tones prior to training resulted in distortions that depended upon the frequency of the tones – i.e., 12- or 3-kHz. Second, in contrast to the very sharp decrease in the gradient of the SB group in Fig. 1, the tonal influence produced a more gradually decelerating gradient. In particular, introduction of the 12 kHz tone before training (Fig. 2) and its continuation throughout extinction resulted in a trend that tended to show suppression at the higher frequency values, while introduction of the 12 kHz tone after training resulted in a fairly flat gradient across extinction testing sessions. A similarly distorted generalization gradient, but in the opposite direction, was also obtained from the group exposed to the 3 kHz tone beginning prior to Pavlovian training (Fig. 3), and a fairly flat gradient emerged when the 3 kHz was introduced after training.

Analysis of the four tonal control groups did not reveal any significant main effects of Background tone, Sequence of tone introduction, or the interaction of these two variables. Likewise, neither the effect of successive extinction Sessions nor the interaction of Sessions with Background and/or Sequence attained acceptable significance. The main effect of Stimuli ($F(4/80) = 3.96, p < 0.01$), and the interactions of Stimuli \times Background tone ($F(4/80) = 6.31, p < 0.0001$), Stimuli \times Sequence ($F(4/80) = 4.07, p < 0.01$), Stimuli \times Background \times Sequence ($F(4/80) = 14.81, p < 0.0001$) were all significant. None of the remaining inter-

actions relating Stimuli with Sessions attained significance. Duncan tests confirmed that the 12B group differed from the 3B group at the 7-, 8- and 11-kHz test values ($p < 0.05$), while none of the 12A–3A comparisons were significant. Within the 12 kHz groups, the 12B group differed from the 12 A group at the 11, kHz test stimulus value only, while the 3B and 3A groups differed from each other at the 7-, 8- and 9-kHz stimuli.

Discussion

The results of Experiment I may be explained by the differential presence of high pitch phantom auditory perception in the salicylate injected subjects. The opposing trends obtained between the SA and SB groups suggest a generalization of tinnitus in both groups with functionally different effects. This finding is consistent with our previous results and expectation. Salicylate-induced perception had contrasting effects on the process of extinction, facilitating it in the SA and prolonging it in the SB situation. It is possible to expect that the closer the external tones are to the perceived pitch of tinnitus, the stronger their effect – i.e., smaller suppression should be observed in the SA situation and greater in the SB. Therefore, the difference between the SA and SB groups should be progressively larger when the frequency of external tones approaches the perceived pitch of salicylate-induced phantom perception.

Examination of the results when salicylate was introduced after training, along with the novel tones,

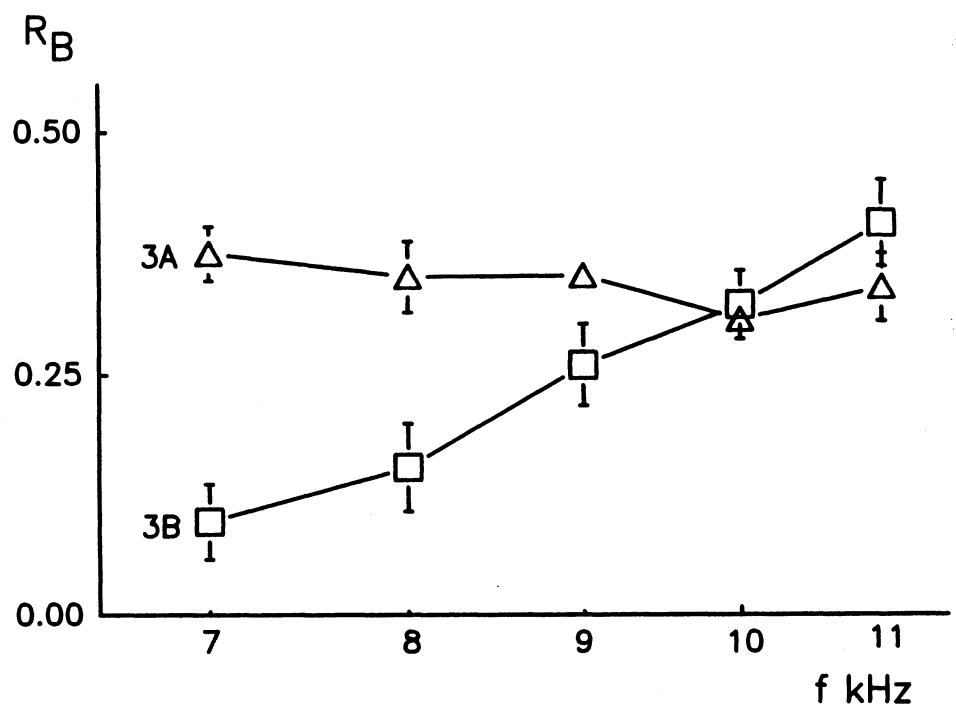


Fig. 3. Generalization gradients for the tonal control groups given continuous presentation of the 3 kHz tone either before (3B, squares) or after (3A, triangles) Pavlovian suppression training.

on the first extinction test, showed little evidence of orientation to the external tones. The internal sound induced by salicylate seemed to provide the SA subjects with sufficient information that the procedure had changed, facilitating extinction, with only some additional influence from external tones. The generalization gradient is rather flat, which is consistent with the postulate that subjects were perceiving an internal sound of high pitch as mentioned earlier.

The possibility that tinnitus was totally or at least significantly masked by external tones can be excluded because the masking of tinnitus, contrary to the masking of external sounds, exhibits flat masking curves (Feldmann 1971), and therefore in such a situation frequency gradients should be absent. The very sharp gradient in the SB group and the flat trend in the SA group argue against a masking hypothesis. The possibility that these gradients are due to a decreased hearing threshold for frequencies closer to 11 kHz is not very likely either, since threshold decreases would act in the same direction for both SA and SB groups, which is contrary to the gradients obtained.

The results of the SB group offer strongest support of the presence of salicylate-induced sound during training along with the aversive US, so that the internal sound acquired conditioned suppression properties. The fact that the SB group suppressed throughout extinction to the two higher frequency values (see Table I) provides a distinct indication that the subjective sound was of a high pitch, probably higher than 11 kHz, and thus generalized best to the external stimuli of 10- and 11-kHz. This interpretation received indirect support from the generalization literature. The training and testing conditions of the SB group are similar to the traditional intradimensional discrimination training procedure (Flower et al. 1980, Guttman and Kalish 1956, Hanson 1959, Ridgman 1975, Switalski et al. 1966). In the SB group, the internal, salicylate induced sound was present during Pavlovian training, and external tones varying along the frequency dimension were presented during generalization testing in extinction. Therefore, the generalization gradient obtained is relatively sharp going from lower to higher frequencies, indicating that training had occurred with perceived frequencies at pitch values greater than 11 kHz.

The tonal control groups are also supportive of this interpretation of salicylate induced distortion of the SB generalization gradient. In particular, the trends in both the 12B and 3B groups showed greater suppression as the novel generalization value approximated the respective values of the additional tones. This

finding, with external physical tones introduced to the subjects, supports the similar interpretation inferring an internal, salicylate induced sound of a relatively high frequency level. However, in light of the discrimination inherent to the 12B gradient, it appears likely that the exact pitch of the salicylate-induced tone may be higher than 12 kHz and probably of a wider band than that represented by the discrete tonal values used presently. The rationale of the tonal control groups was derived from Experiment II of the Jastreboff et al. (1988a) study, and together, these results mimic the predicted effect of the internally, drug induced sound.

In contrast to the SB group, the gradient in the SA group was the product of subjects trained with offset of continuous noise (silence) as the CS, and the phantom perception was introduced concurrently with the presentation of external tones during extinction. This arrangement between training and testing might explain the flat generalization gradient, since in this context the perception of any sound provides information about the change in procedural contingencies, thus facilitating extinction. The observed tendency toward extinction of suppression at higher frequencies might be due to the stronger summation of two signals, phantom and external, when their pitch levels approximate each other.

Finally, the control group results are of interest as well. The introduction of the tones during extinction sessions produced somewhat more suppression than what was expected on the basis of experiments involving the presence of continuous tone during experimental sessions (Jastreboff et al. 1988c). The finding may reflect some secondary aversive properties from training with the US or strong orientation to novel stimuli. One possible explanation is based on differences between apparently analogous SA and Control groups. In the SA group salicylate-induced tinnitus was present all of the time during extinction, and when the external noise was switched off, tinnitus remained present as part of the background. Therefore, we might expect that subjects perceive tinnitus as a part of background which has been associated with safety. As a result, suppression of licking was less and extinction faster. This reasoning is supported by the results from a group of rats trained with a CS consisting of the offset of noise, while a continuous 7 kHz tone was presented during extinction (see Jastreboff et al. 1988c, Experiment II, Fig. 3). These subjects showed rapid extinction as expected.

In contrast, for the Control group of this experiment,

tones were present *only* during the offset of noise. The five different frequencies presented during each experimental session were not part of the background associated with safety, but rather served as novel and continuously changing stimuli. Thus, we might expect some general suppression of licking to occur during the onset of those novel stimuli. The very slight gradient with more suppression at higher frequencies may have resulted from a greater contrast of the background noise and the high frequency tones. Although a white noise generator was used, the higher range of the frequency spectrum is partially attenuated (as evident on the spectral characteristics of the noise presented in Jastreboff et al. 1988c, Fig. 1), due to the frequency characteristics of the loudspeakers. Accordingly, low frequencies might be somehow more alike to the background noise than the higher frequency values, resulting in slightly less suppression at the high frequencies.

EXPERIMENT II.

EVALUATION OF MASKING FROM SALICYLATE- INDUCED PERCEPTION: LOW FREQUENCY EFFECTS

An additional qualification and control for the results of Experiment I involves an evaluation of whether salicylate-induced tinnitus interferes with the perception of external tones in a noticeable way, and, in particular, if phantom perception was sufficient to partially mask external tones. The second and third experiments address this question.

Method

SUBJECTS

A total of 18 male hooded rats, from the same colony as in Experiment I and of initial weight from 307 to 383 g, were used in this experiment.

APPARATUS

The same apparatuses and supporting equipment for daily lick training, suppression conditioning and extinction testing as described for the earlier experiment were used in this experiment.

PROCEDURE

Subjects followed the training and injection sequence outlined in Experiment I, with the following exception: beginning with the first day of lick training, subjects were exposed continuously, in the home cage and in the experimental chamber, to a 65 dB SPL 5 kHz tone imposed upon the background noise. Accordingly, during acclimation exposure and conditioned suppression training sessions, the background noise went off for 1 min, leaving only the 5 kHz tone present. After reaching the suppression criterion, all subjects were given five generalization tests during which frequencies of the tone present during the CS probes were varied among 4-, 5-, 6- and 7-kHz. Groups of six subjects each were assigned to salicylate before (SB) or after (SA) treatments or saline control (C) procedures, as described for Experiment I. The same ratio measure, R_B , defined above was also computed for this experiment.

Results and Discussion

Reliable within-subjects generalization with maximum suppression at the 5 kHz value emerged during testing for all groups. Figure 4 depicts group mean gradients of generalized suppression for each of the salicylate treatment conditions and the control subjects. There were no significant effects from differences in salicylate administration in the three groups. As Fig. 4 reflects, the analysis of suppression ratios indicated main effects from variation of stimulus Frequency values in all groups ($F(3/45)=6.70$, $p<0.01$). The effect of progressive extinction Sessions ($F(5/60)=3.41$, $p<0.05$) was also found, and a Group \times Frequency interaction effect ($F(6/45)=2.39$, $p<0.05$) emerged. In light of Fig. 4, this interaction may be attributed more to the modest switch in maximum levels of suppression at particular generalization values (e.g., the control group's responding at 4- and 5-kHz stimuli), rather than any clear salicylate effects. The results of the Duncan tests support this interpretation since the C and SB groups differed at 4 kHz, while the C and SA groups differed at 5 kHz, and the relative position of the control group to the respective salicylate groups was opposite.

These results indicate that generalization to values away from 5 kHz, the frequency presented during training, was very systematic across all groups, suggesting that the external tone is dominant in this paradigm. The addition of salicylate induced auditory

experience in the SB and SA groups did not alter the shape and extent of generalization relative to controls. None of the individual session analyses indicated any influence of salicylate treatment. Thus, the auditory perception induced by salicylate did not appear to mask or otherwise interfere with the discrimination of external stimuli related to acquired control by CS frequency.

EXPERIMENT III. EVALUATION OF MASKING FROM SALICYLATE-INDUCED PERCEPTION: HIGH FREQUENCY EFFECTS

Experiment I indicated that the pitch of salicylate-induced phantom perception was around or above 11 kHz. During training and testing in Experiment II we used tones of lower frequencies to provide clear separation between external physical from internal, phantom tones. In Experiment III we investigated the possible influence of salicylate-induced phantom perception on external tones of similar frequency range. In addition, the two procedures of Experiment III attempted to specify more clearly the potential masking role of the tonal stimulus in relation to the continuous noise background.

Method

SUBJECTS

A total of 48 pigmented rats were deprived of water and housed under similar arrangements as in Experiment I. Subjects' weight at the start of experimentation ranged from 327 g to 403 g.

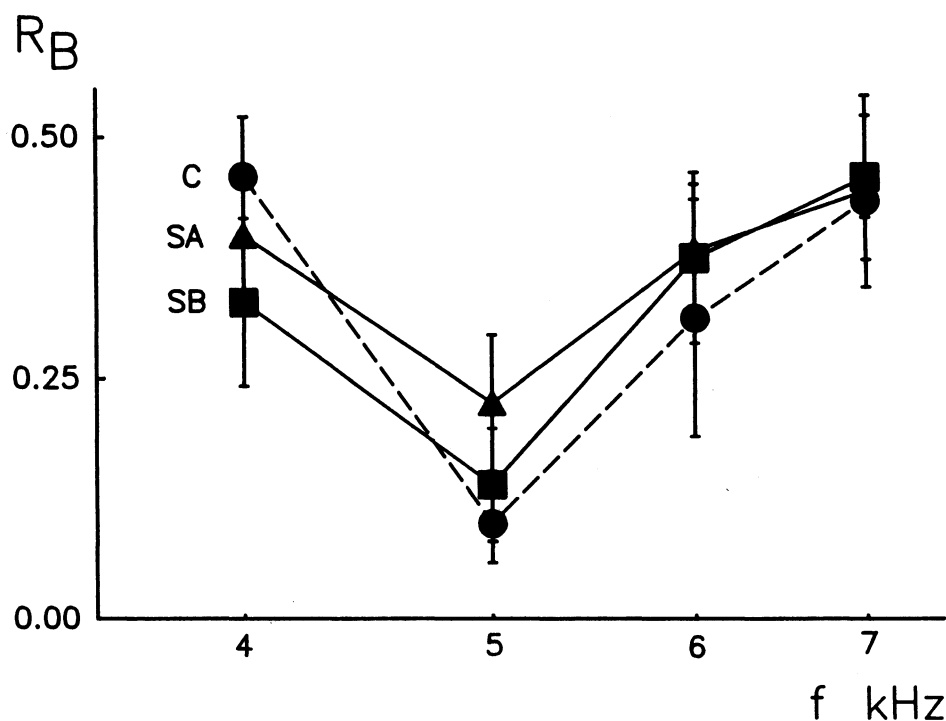
APPARATUS

The same apparatuses and supporting equipment for daily lick training, suppression conditioning and extinction testing as described for Experiment I were used in this experiment.

PROCEDURE

Following the procedures outlined in Experiments I and II, 24 subjects were exposed from the time of water deprivation to the same continuous wide band background noise with a superimposed discrete tone of 10 kHz at 65 dB. During CS probes, offset of the background noise left the 10 kHz tone on alone for the 1 min duration. An additional 24 subjects received exposure to the background noise only, without the additional tone. Training under the second procedure involved a CS consisting of offset of the background noise accompanied by the onset of the 10 kHz tone. After 1 min the 10 kHz tone went off with the onset of the background noise. The suppression training and generalization testing procedures for both groups

Fig. 4. Group mean generalization of suppression and related SEM_s to varying frequency values presented in extinction, after training to a CS consisting of offset of background noise and continuation of a 5 kHz background tone. Each mean represents 6 subjects, 5 days of extinction, 4 probes per day. All symbols as in Fig. 1. Note clear generalization to 5 kHz and the lack of any clear differential effect of salicylate administration.



followed the same sequence as in Experiment I. Generalization test stimuli of 7-, 8-, 9-, 10- and 11-kHz (all at 65 dB) were presented once in each session, and the order of presentation was counter-balanced across subjects. Within each background stimulus contingency, subjects were assigned to one of the three salicylate treatments ($n=8/\text{group}$) described in Experiment I, and the same measure of performance, R_B , was computed for this experiment as well.

Results and Discussion

TONE + NOISE BACKGROUND

The group mean gradients of the response ratio, R_B to each tonal frequency value in subjects trained with the 10 kHz tone superimposed on the background noise are shown in Fig. 5. Reliable gradients of generalization were obtained from individual subjects as well, with the only noticeable effect from salicylate treatment manifested at the 8 and 9 kHz frequency values of the generalization curve.

Analysis of the generalization data indicated the presence of some effects from the Group treatment ($F(2/21)=4.41, p<0.05$), which may reflect a somewhat modest salicylate effect. Duncan comparisons indicated differences ($p<0.05$) between controls and the other two groups at 7-, 8- and 9-kHz frequencies. The SB and SA groups did not differ. The group effect indicates a possible salicylate influence in which the presence of tinnitus during extinction has some suppressing effect

while its presence during training is irrelevant. However, the important point is that the salicylate effect was nondifferential with respect to the sequence of injections in conditioning. The expected effects of successive testing Sessions ($F(4/84)=2.87, p<0.05$), and generalization testing Frequency ($F(4/8)=9.38, p<0.0001$) were obtained, while none of the interactions attained acceptable significance.

NOISE BACKGROUND

Figure 6 shows the group results for subjects trained with the background of noise only, and the CS comprised the offset of the noise and onset of the 10 kHz tone. There is a clear indication of gradients centred on 10 kHz without any noticeable group differences. Analysis of the generalization data indicated that only the effect of test Frequency was significant ($F(4/84)=4.11, p<0.01$), and Duncan test showed that for each extinction session the effect occurred across group differences (all $ps<0.05$). These results suggest that the presence of the tone only during CS periods tended to produce sharp stimulus control without differences due to salicylate treatments.

GENERAL DISCUSSION

Only when the external tones were removed from direct association with shock in Experiment I was there

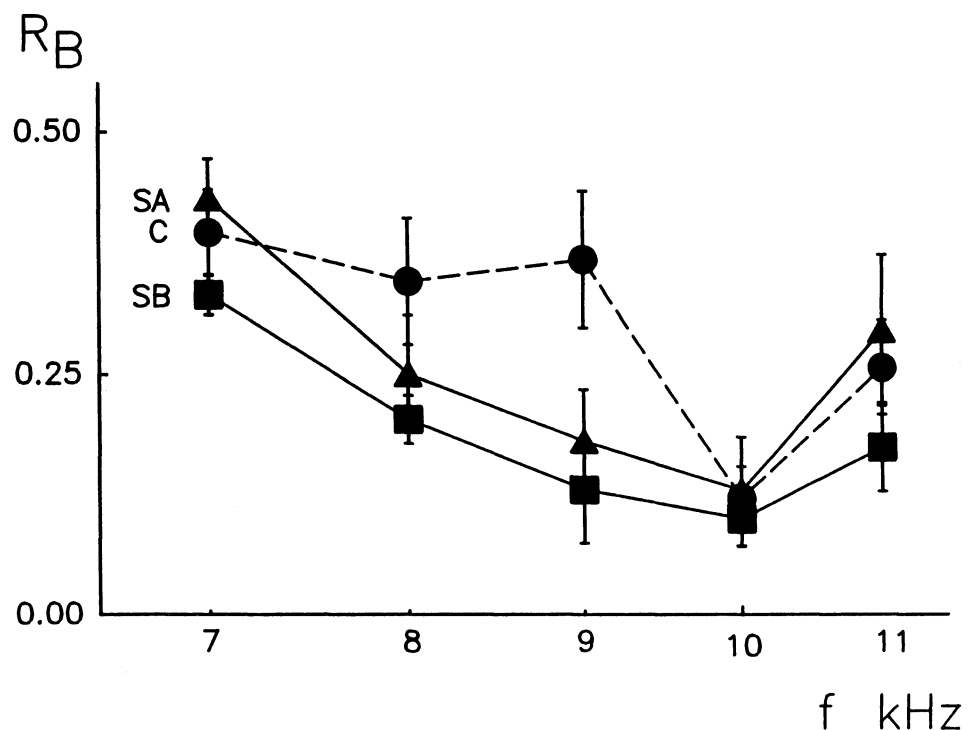


Fig. 5. Mean suppression ratios to generalization stimuli after training with the 10 kHz tone present with background noise during the CS periods in Experiment III. Each mean represents 8 subjects, tested daily with 5 frequency values over 5 extinction days.

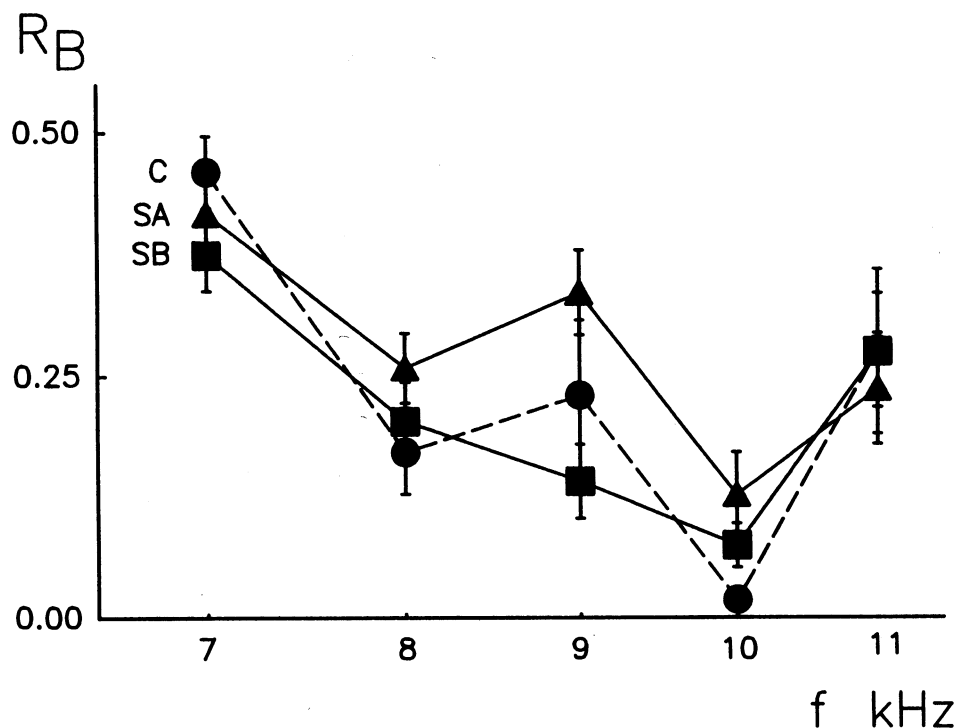


Fig. 6. Mean generalization of response suppression for the groups in Experiment III trained with the 10 kHz tone presented during CS periods only. All other descriptions as in Fig. 5.

evidence of the effect of salicylate-induced phantom perception on the generalization process. That is, the salicylate-induced tinnitus associated with the US during training in the SB group showed profound generalization to the higher frequency values of the novel tone in extinction. When the subjective internal auditory experience was introduced after training in the SA group, the resulting gradients were broad and showed little overall suppression, indicating that salicylate-induced experience acquired safety value from the subjects and dominated over perception of external tones. The observation that weaker suppression existed for higher frequencies is consistent with the postulate of generalization from high-pitched phantom sound. These results contrast with data obtained in Experiments II and III where the paradigm was aimed at creating dominance of external tones. The external tones were present during training and even if tinnitus was introduced at the same time in the SB groups on the basis of human data on salicylate-induced tinnitus, it is possible to expect that tinnitus was easily dominated (McFadden and Plattsmier 1983). Consequently, Experiments II and III show a lack of any marked influence exerted by internal, phantom sound on perception of external tones. The dominant effect of external tones acquired through conditioned suppression training was clear in both experiments. The evidence of frequency generalization was found consis-

tently in individual subjects, regardless of the background auditory context employed or the stimuli presented during extinction testing. Indeed in Experiments II and III, the effects of stimuli were consistently present during the CS periods.

No masking influence from the salicylate-induced auditory phantom perception on frequency generalization was observed in these experiments when the external tones were associated directly with conditioned suppression training. In Experiments II and III, frequency generalization was obtained from all groups. Within each respective level of suppression, frequency control was relatively comparable between groups, suggesting that any auditory influence emanating from the effects of salicylate injections was not sufficient to mask or distort the frequency effect in a pronounced manner. Collectively, these experiments show that auditory phantom effects induced by salicylate resulted in contrasting generalization gradients depending on the time of introducing salicylate and the used paradigm, strongly suggesting that subjects perceived high-pitched phantom sound. By expanding the range of analysed frequencies to higher values and using the paradigm of Experiment I it should be possible to determine the range of perceived pitch of salicylate-induced tinnitus. These data broaden the scope of the animal model of tinnitus, and they add to the options for applications of the model.

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