Habituation of the rat's respiratory response to auditory stimulation during sleeping and waking

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The rat's respiratory response to auditory stimuli is described. Stimulation within a session consisted of 10 16-kHz tones of 60 dB SPL and 3 16-kHz tones of 80 dB SPL. Form and habituation of the respiratory response were not affected by the rat's state of vigilance: Onset and offset of auditory stimulation evoked a respiratory pause whether the rat was awake or in slow-wave sleep. The response displayed short-term, but not long-term, habituation: It disappeared within a session, but showed complete recovery when the stimulation was repeated 1 week later. The data are discussed with respect to the question of whether the respiratory response evoked by auditory stimulation constitutes an orienting response (OR) within the OR concept.

The present study describes the rat's respiratory response following auditory stimulation during slow-wave sleep (SWS) and during the awake state to answer the following questions: (1) Is there short- or long-term habituation during SWS and the awake state? (2) Is this response an OR in terms of OR theory?

A novel stimulus of moderate intensity can cause an orienting reflex (OR) in animals and humans, as first described by Pavlov (1927) and Sokolov (1963). Peripheral ORs, such as changes in heart rate and respiration, accompany central information processing of an organism and are thought to lower sensory thresholds and enhance intake of information. One of the main characteristics of the OR is that habituation of the response will occur upon repeated presentation of the same stimulus. Sokolov hypothesized that elicitation of the OR is mediated subcortically, whereas habituation of the response requires cortical or hippocampal activity. Since the nature of cortical activity during the sleeping and awake states is quite different, the nature of the OR and its habituation (which can be considered a form of learning) could be expected to be different during waking and sleeping. This appears to be true for the cardiac response to tones in humans (Berg, Berg, & Graham, 1971; Berg, Jackson, & Graham, 1975).

However, there are contradictory experimental findings, which raise the question of whether habituation can occur during sleep. Jung (1954) and Sokolov and Paramanova (1961) reported habituation in humans during sleep, and Johnen (1987) found habituation of the rat's heart-rate response during SWS.

Other authors have failed to find habituation during

sleep (Firth, 1973; Johnson, Townsend, & Wilson, 1975; Williams, Hammack, Daly, Dement, & Lubin, 1964). Johnson and Lubin's (1967) data indicated that sleep blocked habituation in the case of autonomic variables, which led the authors to conclude that "sleep and habituation are incompatible," at least for the variables they studied. Williams (1973), in summarizing studies of habituation during sleep, stated that "with a few exceptions . . . the majority of controlled studies appear to rule out sleep learning" (p. 39).

Comparatively few authors have described an animal's respiratory response to auditory stimulation. Among them are Corbeille and Baldes (1929) and Criborn (1970), who reported changes in the breathing frequency of frogs, dogs, and mice following auditory stimulation. Such (1959) examined the influence of sound on the rabbit's heart rate and breathing rate. The data from these studies were not evaluated in a quantitative manner, however, so they provide only vague information about responses to auditory stimulation. The present study offers a more differentiated method for evaluating the rat's auditory-evoked respiratory response, using a breath-to-breath analysis.

METHOD

Subjects

The subjects were 23 naive male Wistar rats with a mean body weight of $331 \text{ g} \pm 8.3 \text{ g}$ (SEM). The rats were housed individually at 21°C , with food pellets and water ad lib on a light/dark (LD), 12 h:12 h schedule, with lights on at 8 a.m. Two groups of animals were formed according to their time of testing: 8 rats were tested between 8 a.m. and 12 noon (SWS) and 15 rats between 8 p.m. and 10 p.m. (waking state).

Apparatus and Stimuli

Respiration. The unrestrained animals were measured inside a transparent Plexiglas cylinder 15-cm diameter, 25-cm height. Their breathing movements were picked up by a Bell & Howell pressure

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transducer with a frequency range from 0 to 100 Hz. The transducer was connected to the cylinder by a flexible rubber tube (whole-body plethysmography), and its output was amplified by a Bell & Howell bridge amplifier. Thus, the animals could move freely within the cylinder. Fresh air was provided by a continuous, moderate airflow.

Electrocorticogram (ECoG). Electrical brain activity was measured via an FM miniature telemetry system, which is described elsewhere in detail (Neuhaus & Borbely, 1978). Briefly, two gold-plated screws chronically implanted in the skull (one positioned above the right parietal cortex, the other above the frontal cortex) served as electrodes. They were connected via stainless steel wire to a miniature FM radio transmitter placed on the rat's head. The omitted signals were received by three antennas surrounding the animal, each at a distance of 1.5 m. The ECoG was bandpass filtered (0.5–30 Hz), amplified, and displayed on a Tektronix dual-beam-storage oscilloscope. For technical reasons, no quantitative on-line analysis of ECoG frequency could be carried out. It was possible, however, to distinguish different states of vigilance during the experiment by visually scoring the ECoG amplitude displayed on a Tektronix dual-beam oscilloscope. A sleep stage was judged to be SWS when the following three criteria were fulfilled: High-voltage, low-frequency activity in the ECoG, regularity of respiration, and motionlessness of the rat (slight body movements were seen as artifacts in the respiration signals). Figure 1 shows synchronously registered ECoG signals and breathing movements of a rat during waking and sleeping. Respiration and ECoG data were stored simultaneously on FM tape and writing paper (Gould 2200 two-channel recorder) for further evaluation.

Acoustic stimulation. Stimulus intensity inside the Plexiglas cylinder (2 cm above the bottom) was measured with a ¼-in. Brüel & Kjær microphone and a Hewlett-Packard frequency analyzer. Background noise was below 40 dB SPL (re: 20 µPa). Stimuli were generated by a Toellner 7706 frequency generator, attenuated with a Hewlett-Packard 350-D attenuator and amplified by a Hewlett-Packard 467 A amplifier. Their durations and rise and fall times were controlled by an electronic pulse generator via the frequency generator's VCA input. The loudspeaker (commercially available, high-frequency, high-fidelity) was positioned about 15 cm above the rat's head in the upper part of the Plexiglas cylinder. All experiments were carried out in a special anechoic and soundproof room.

Procedure

Experiments with the rats in two different states of vigilance were carried out with rats in SWS in Experiment 1 and with rats in the awake state in Experiment 2. Each experiment comprised two sessions. There were 13 16-kHz tones in Week 1, and they were repeated in Week 2. The tones had a duration of 2 sec, an inter-stimulus interval (ISI) of 60 sec, and a rise/fall time of 5 msec. The sound pressure level of Stimuli 1–10 was 60 dB and that of Stimuli 11–13 was 80 dB.

Experiment 1 (SWS). Eight animals were anesthetized with a mixture of Ketavet (Parke-Davis, 100 mg/kg) and Rompun (Bayer, 2.3 mg/kg) in order to fix electrodes and FM transmitter. One week later, daily training sessions were begun. On 5 consecutive days, each animal was placed into the Plexiglas cylinder for 15 min twice a day without any stimulation. This served to shorten the latency of sleep onset by familiarizing the animals with the experimental situation. Subsequent to this training phase, stimulation experiments were begun. A rat was placed in the Plexiglas cylinder within the first 4 h of the light (L) phase, because total sleep time and SWS density are highest at this time (Borbely, 1982). The stimuli were presented only when the animal's sleeping state was identified as SWS using the previously mentioned criteria. If the animal woke up during the experiment or switched to another sleeping state (e.g., REM sleep, characterized by a low-voltage, high-frequency ECoG

and a curled-up, motionless body position with eyes closed), the stimulation was stopped until SWS reappeared. This was rarely the case, however. The number of changes in the state of vigilance per session and animal ranged from 0 (i.e., 13 stimuli with an ISI of 60 sec were delivered without interruption) to 5 (i.e., there were 5 ISIs longer than 60 sec), with a mean of 2.

Experiment 2 (awake state). Fifteen naive animals were used. The procedure was similar to that of Experiment 1, except that no training was carried out before the experiment. Thus, the animals in the awake state were not as familiar with their surroundings as were those in SWS (for more on this point, see Discussion). The rat was placed in the measuring cylinder at the beginning of the dark (D) phase, when total sleep time and SWS density are low (Borbely, 1982). All experiments were carried out in dim red light (40-W red bulb, approximately 12 lx). Stimulation began after an adaptation period of about 10 min. Normally, the rat sat with eyes open in a sphinx-like position. No stimuli were delivered during sniffing and exploratory behavior. Although no ECoG measurements were taken in this experiment, behavioral observations showed that the animals were in the awake state during at least 90% of the time they spent in the cylinder.

Evaluation of Data

Preliminary experiments had shown that the rat's respiratory response could be characterized by a lengthened breathing cycle, that is, a respiratory pause following the breathing cycle immediately before stimulus onset and offset. Accordingly, a breathing cycle was defined as the horizontal distance between two maxima (end of inspiration) of the breathing curve (see Figures 1 and 2 for original data). This respiratory pause is expressed in percent response magnitude, which represents the percentage of increase in the duration of the breathing cycle following stimulus onset or offset as compared with the cycle before onset or offset. For an example, see Figure 2, which shows the registration of a sleeping rat's respiratory response to auditory stimulation. In addition to a respiratory pause, a diminished breathing amplitude was noticed following stimulation. Because this response occurred in a rather unsystematic manner, however, these results are not reported in this study.

Statistical Analysis

Time courses of responses to Stimuli 1–10 were analyzed using a polynomial regression based on an analysis of variance. Comparisons of two means were made with Student's two-tailed *t* test for either paired or unpaired values. Data not normally distributed were evaluated with the nonparametric Wilcoxon *U* test and the Wilcoxon matched-pairs signed-ranks test. All values are expressed as mean $\pm$ SEM unless stated otherwise.

RESULTS

Figure 1 shows synchronously registered ECoG and respiration of an animal during sleeping and waking. The high-voltage, low-frequency ECoG together with regular breathing signals was scored to be SWS, whereas a low-voltage, high-frequency ECoG with irregular breathing signals was scored to be the awake state. The rat's respiratory response to auditory stimulation was similar during sleeping and waking, and can be characterized by a respiratory pause following both tone onset and tone offset. Figure 2 shows the auditory-evoked respiratory response of a sleeping rat. The respiratory pause following tone offset is shown to be larger than that following tone onset. The mean breathing frequency immediately before stimulation was 80 ± 29.5 breathing cycles/min for

Figure 1. Simultaneously recorded electrocorticogram (ECoG) and breathing movements of a rat during a sleeping state that was judged as slow-wave sleep and in the waking state. Note that during sleep there is a high-voltage, low-frequency ECoG together with regular breathing movements, and in the waking state there is a low-voltage, high-frequency ECoG together with irregular breathing movements. Inspiration upward.

the rats in the awake state and 86 ± 12.8 breathing cycles/min for the sleeping rats. The difference between the two means was not significant (Student's *t* test), whereas the standard deviations were significantly different [$F(443, 216) = 5.3, p < .001$]. This indicates that respiration was more regular during sleep than during the awake state.

Experiment 1 (Sleeping Rats)

Respiratory response to tone onset. As shown in the upper part of Figure 3, tone onset was followed by a respiratory pause consisting in a 10%–30% increase in breathing cycle length compared with the preceding breathing cycle. In Week 1, this respiratory pause showed no significant response decrement across Stimuli 1–10 [$F(1,75) = .46, p > .05$, for linear-trend analysis; $F(2,74) = .36, p > .05$, for quadratic trends]. The figure shows that in both weeks the response to Stimulus 1 was considerably smaller than that to Stimulus 2, because only half of the animals responded to the onset of the first tone with a respiratory pause, whereas the other half responded

with an acceleration. A trend analysis considering only Stimuli 2–10, however, did not yield significant results. In Week 2, the regression analysis showed significant linear [$F(1,88) = 9.5, p < .005$] and quadratic [$F(2,87) = 6.4, p < .005$] trends of habituation across Stimuli 1–10. The 80-dB tones following the 60-dB tones had no effect on the habituated response: There were no significant differences between responses to Stimulus 10 (60 dB) and Stimulus 11 (80 dB).

Respiratory response to tone offset. Short-term habituation of the respiratory pause following tone offset can clearly be seen in the response decrement across the 10 60-dB tones, which are shown in the lower part of Figure 3. In Week 1, response magnitude decreased from 48% (first 60-dB tone) to 10% (last 60-dB tone). There are both linear [$F(1,74) = 21.2, p < .001$] and quadratic [$F(2,73) = 13.9, p < .001$] trends of habituation. One week later, the response had recovered completely but again showed short-term habituation, which can be seen in the decrease from 57% (first 60-dB tone) to nearly zero (last 60-dB tone), with $F(1,75) = 26.3$ for linear and $F(2,74) = 25.7$ for quadratic trends of habituation. The 10 60-dB tones were followed by 3 80-dB tones (Stimuli 11–13). These tones caused no recovery of the habituated respiratory response, that is, the response remained near zero. Tone offset evoked a larger respiratory pause than did tone onset [for Week 1, $R(8) = 3, p < .05$; for Week 2, $R(8) = 0, p < .05$].

Experiment 2 (Rats in the Awake State)

Respiratory response to tone onset. Significant linear [$F(1,142) = 12.9, p < .001$] and quadratic [$F(2,141) = 9.61, p < .001$] trends of habituation to tone onset were seen in Week 1, but not in Week 2 [$F(1,130) = 1.3, p > .05$, for linear trends; $F(2,129) = 1.7, p > .05$, for quadratic trends]. The results for both weeks are shown in the upper part of Figure 4.

Respiratory response to tone offset. The responses to tone offset in Weeks 1 and 2 are shown in the lower part of Figure 4. Similar to Experiment 1, there was short-term, but no long-term habituation. Short-term habituation to tone offset was significant in Week 1 [$F(1,139) = 67.6, p < .001$, for linear trends; $F(2,138) = 47.1, p < .001$, for quadratic trends] as well as in

Figure 2. Original data showing a sleeping rat's respiratory response to a 60-dB, 16-kHz tone. Both stimulus onset and offset are followed by a respiratory pause. Inspiration upward. Pre = pre-stimulus level; Post = poststimulus level.

Figure 3. Respiratory pause following onset (top) and offset (bottom) of 16-kHz tones (sleeping rats, $n=8$). Trials 1-10: 60 dB SPL; Trials 11-13: 80 dB SPL.

Week 2 [$F(1,125) = 24.3$, $p < .001$, for linear trends; $F(2,124) = 18.9$, $p < .001$, for quadratic trends]. The first 60-dB tone was followed by a response magnitude of 82% in Week 1 and by one of 87% in Week 2, which indicates that the habituated response had completely recovered within 1 week without any signs of long-term habituation. In both weeks, tone offset of the first 60-dB tone caused significantly larger response magnitudes than tone onset [for Week 1, $R(15) = 3$, $p < .001$; for Week 2, $R(10) = 0$, $p < .01$]. Just as with tone onset, the offset response to the 80-dB tones was not different from that to the 60-dB tones. Thus, the 20-dB increase following 10 presentations of 60-dB tones evoked no change in responsivity independent of the animals' state of vigilance.

DISCUSSION

The results show that (1) the rat's respiratory response to moderate-intensity pure tones consists in a respiratory pause following tone onset and offset, (2) there is habit-

uation of this response within a session (short-term habituation) but not between sessions (long-term habituation), and (3) short-term habituation occurs during sleeping as well as during waking. Although there were differences in responsivity between the two states of vigilance (a higher response magnitude during the awake state), these differences could also be due to differences in familiarity with the test environment. As previously mentioned, only the sleeping rats had been made familiar with their surroundings prior to testing. There were no differences between the two groups in rate of habituation. This is in accordance with the results of Johnen (1987), who found clear habituation of the sleeping rat's heart-rate response to auditory stimulation. It thus appears that the rat's habituation to auditory stimulation does not depend on its state of vigilance. The 80-dB stimuli following the series of 10 60-dB stimuli failed to evoke an additional OR once habituation had occurred. Their lack of effect when presented at the end of a series of 60-dB tones could be due to stimulus generalization.

As already mentioned, sleeping rats did not respond to the first onset of auditory stimulation (Figure 3). This was

Figure 4. Respiratory pause following onset (top) and offset (bottom) of 16-kHz tones (rats in the waking state, $n=15$). Trials 1-10, 60 dB SPL; Trials 11-13, 80 dB SPL.

not due to nonresponding, but to the fact that half of the animals responded with a respiratory pause, whereas the other half responded with an acceleration, that is, breathing-cycle length became shorter.

Caspers, Grueter, and Lerche (1958) found that 60 dB at 16 kHz was an intensity below the rat's awake threshold. This was confirmed by the present experiments, since this intensity evoked no behavioral (motor) arousal during SWS, despite the described respiratory responses. Stimulus intensities in the range of 60 dB are appropriate for the evocation of an OR (Sokolov, 1963). According to Sokolov, an OR is sensitive to habituation, and the response should be similar to turning a stimulus on or off. As described above, the rat's respiratory response to auditory stimulation was sensitive to habituation during sleeping and waking. Onset as well as offset of the tones evoked a respiratory pause. Barry (1977b) examined, in humans, respiratory responses to 1-kHz tones of 20–50 dB SPL. He reported a 10%–20% increase in respiratory period at stimulus onset, which habituated upon repeated stimulation (Barry, 1977a, 1977b, 1982). In terms of Barry's proposed four-way fractionation of response indicators, the respiratory OR is controlled by the "novelty register" and therefore is sensitive to habituation. Thus, the present data are in accordance with Barry's as well as Sokolov's OR theory, and the form of the rat's respiratory response to 60-dB 16-kHz tones is similar to a human's respiratory OR to 20–50-dB 1-kHz tones.

A direct comparison can be made between Barry's (1977a, 1977b, 1982) results and those of the present study, because the rat's sensation level at 16 kHz is similar to that of humans at 1 kHz, a commonly used frequency in OR experiments (see Kelly & Masterton, 1977, for the rat's hearing threshold, and Zwicker & Heinz, 1955, for that of humans). A plausible explanation for the respiratory pause following low-intensity auditory stimulation was first given by Woodworth (1936), who wrote: "Sudden stimuli will make the subject 'catch his breath.' If he is listening to a faint sound, arrested breathing eliminates disturbing respiratory sound" (p. 260).

Since habituation of the rat's respiratory OR was seen during both sleeping and awake states, our results are in contrast to most of the findings in experiments with humans, where habituation is a function of the subjects' state of vigilance. Johnson and Lubin (1967), for example, came to the conclusion that habituation and sleep were incompatible. They found that the average respiratory response to 1-kHz tones near threshold was a slowing in breathing rate, regardless of the state of vigilance. This response habituated during the awake state, but returned with sleep onset and remained during sleep. The authors reported a response magnitude of approximately 10% for humans, whereas the rat showed a response magnitude of more than 80% during the awake state and one of about 50% during SWS.

If habituation is considered to be a form of learning, it appears that SWS does not interfere with this form of learning in the rat.

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