

## ALTERED INFORMATION PROCESSING DURING SLOW WAVE SLEEP IN RATS

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### INTRODUCTION

Neurophysiological studies have indicated that the transfer of sensory information through the specific nuclei of the thalamus depends on the sleep-wake state. The transfer-ratio concept of Coenen and Vendrik (1972) states that this ratio between thalamic input from the sensory organs and thalamic output to the cortex is equal to 1.0 during wakefulness, whereas it is reduced to 0.4 during slow wave sleep (SWS). A transfer-ratio of 1.0 thus means that all sensory information will reach the cortex as the input in the thalamus is equal to the output of the thalamus. In contrast, during SWS this 'thalamic gate' is closed to a certain extent and only a part of the sensory information is available to the cortex (Coenen, 1995). The question arises whether this gating mechanism influences information processing during SWS.

A complex form of information processing is excitatory learning: an initially neutral stimulus (CS: e.g., a tone) is by repeated paired presentation associated with a biologically relevant unconditioned stimulus (US: e.g., a footshock). This conditioning can be analysed by means of measuring the behavioural response to the stimuli. After several pairings the CS will predict the occurrence of the US and will elicit a similar response as the US. Only a few animal studies have investigated the possibilities for and mechanisms underlying excitatory conditioning during sleep. Excitatory associative learning was found possible during REM sleep, but not during SWS (Hars et al., 1985; Hars & Hennevin, 1987; Hennevin et al, 1995).

To our knowledge only one study has addressed the capacity of the sleeping rat brain for inhibitory conditioning, i.e., the CS predicts that the US will not be presented (Roobol et al, 1996). That study investigated extinction during SWS, a simple form of learning, which can be defined as "learning to respond no longer to previously conditioned stimulus" and found that extinction can take place during REM and SWS, but is less efficient during the latter (Drinkenburg et al., 1996; Drinkenburg et al., in prep; Roobol et al., 1996). More complex inhibitory learning can be studied in the rat as well: after several presentations of an initially neutral CS without an US the CS becomes a 'conditioned inhibitor'. Whether or not a stimulus is a conditioned inhibitor can be tested (Mackintosh, 1983): if a subject is pre-exposed to the to-be-conditioned stimulus (e.g., a tone), this will retard subsequent excitatory conditioning of that stimulus to an US (e.g., a mild footshock). The present study will use this retardation principle to investigate the effects of repeated presentations of a tone without consequences during SWS. Next, after the tone has been associated with the

presentation of a footshock during wakefulness, the behavioural expression of earlier inhibitory learning during SWS will be measured by analysing changes in operant responding following presentation of the tone. Our hypothesis is that during SWS the perception of the tones will be altered, causing a sub-optimal processing (learning) of the pre-exposure tones during SWS and subsequent reduced capacity of the tones to retard the acquisition of an association with the footshock. This will be expressed in changes in operant behaviour after presentation of the tone; latency to restart pressing and post-stimulus lever press behaviour.

## METHODS

*Animals.* Sixteen male Wistar rats with a mean ad libitum weight of 351.6 gram (range 260-450 gram) were individually housed in a temperature controlled room ( $23\pm 1^\circ\text{C}$ ), in type 3 macrolon cages. A 12/12-hour light dark cycle was maintained throughout the experiment (lights on at 9.30 a.m.). After operation food deprivation down to 85% of the rat's free feeding weights was started, while water was continuously available. At the start of the behavioural training animals were approximately five months old.

*Surgery.* To classify sleep-wake states the animals were implanted with a permanent tripolar EEG electrode set (Plastics One, MS 333/2-A) and a bipolar electromyography (EMG) electrode set (Plastics One, MS 307/71) under complete anaesthesia (45 ml/kg Narcovet i.p. combined with 75 ml/kg Hypnorm i.m.). Additionally, the local anaesthetic marcaine was injected around incision. Stereotaxic coordinates with skull surface flat and with respect to bregma were 2.0 AP, -3.5 LAT, and -6.0 AP, -4.0 LAT, for the two active EEG electrodes respectively. The reference electrode was placed over the cerebellum. Both EMG electrodes were placed subcutaneously in the nuchal muscles. Following the operation the animals were left undisturbed for a recovery period of two weeks.

*Behavioural procedure.* All experimentation was carried out in operant chambers which were adapted to allow parallel EEG/EMG recording (filtering:  $1\text{ Hz} < \text{EEG} < 100\text{ Hz}$ ;  $10\text{ Hz} < \text{EMG} < 1000\text{ Hz}$ ). To optimize the chance of obtaining periods of SWS next to behavioural activity, testing was planned in the middle of the light period. The experiment consisted of the following phases:

- 1) - Fixed-Ratio 30 (FR-30) training: In order to obtain a stable operant behaviour, which will be used to measure the suppressive capacity of the stimulus, animals were trained to press a lever on a Fixed Ratio 30 schedule of reinforcement (i.e., a fixed number of 30 consecutive lever presses are needed before a food pellet is given).
- 2) - Pre-exposure phase: In this phase the animals received ten auditory stimuli (CS: random  $5\pm 3$  s. duration; 12 kHz frequency; 51,7 dB(A) intensity) exclusively during either SWS or active wakefulness, depending on the experimental condition to which (groups were weight-matched) they were allocated. The purpose of this phase was to pre-expose the animals to the to-be-conditioned stimulus.

3) - Excitatory conditioning phase: In this phase all animals were presented three pairings of the auditory stimulus (CS) with a mild scrambled foot-shock (US: 1 s. duration; 0.5 mA intensity) to give the auditory stimulus the potential to suppress ongoing operant behavior in the following testphase.

4) - Test-phase: In this phase it was tested whether the CS-US associative strength had been differentially influenced by pre-exposure of the CS during SWS or wakefulness (see pre-exposure phase). The CS was presented to both groups on the fourth trial, immediately after the fifteenth leverpress. Latency after stimulus onset (latency to restart responding after stimulus onset), pre-stimulus leverpress behaviour (mean inter leverpress latency before stimulus onset) and post-stimulus leverpress behaviour (mean inter leverpress latency from leverpress 16 to leverpress 29) were analysed by means of ANOVA, where appropriate after Log transformation of data.

## RESULTS

In Figure 1 leverpress behaviour during trial 3, the trial preceding the testtrial, is shown. It can be seen that leverpressing was done in a consistent, continuous way and was not differing between the two experimental conditions. Leverpress behaviours during the testphase are shown in figure 2.

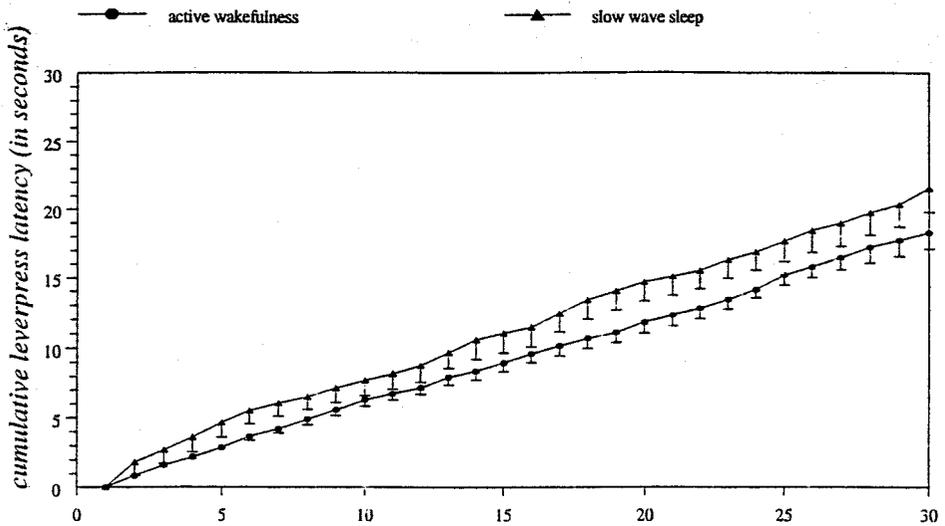


Figure 1:  
Mean cumulative leverpress latency (in seconds) during trial 3 (preceding the test-trial) with SEM for each subsequent leverpress within the FR-30 schedule. Filled circles indicate results of the active wakefulness group; Triangles indicate results of SWS group.

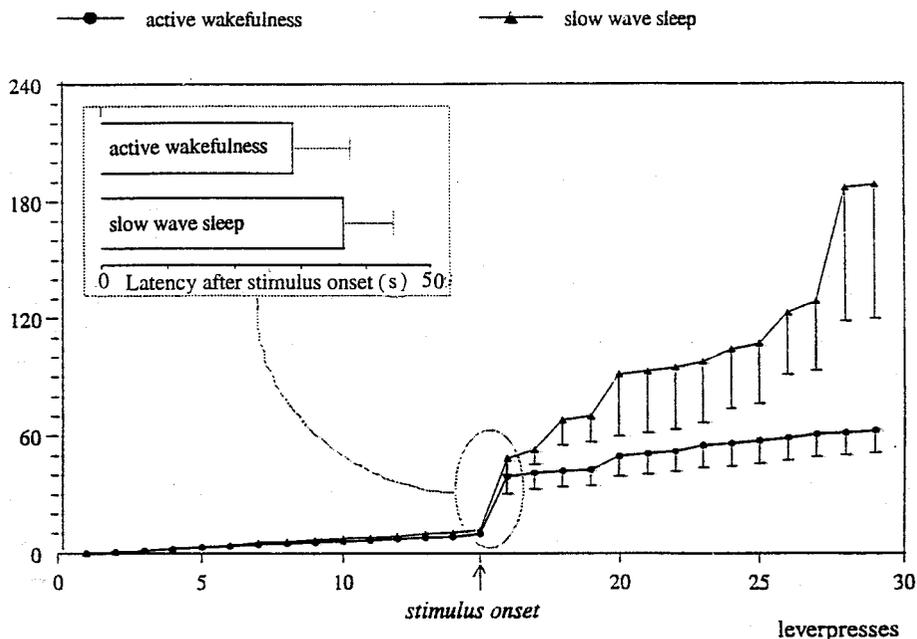


Figure 2: Mean cumulative leverpress latency (in seconds) during the testtrial with SEM for each subsequent leverpress within the FR-30 schedule. Filled circles indicate results of the active wakefulness group; Triangles indicate results of SWS group. Please note the changed range in Y-axis as compared to Figure 1. In the fill out, mean latencies (in seconds) to restart leverpressing after stimulus onset are displayed plus SEM.

With respect to pre-stimulus leverpress behaviour (first 15 presses) there were no differences between the two groups ( $F=1.549$ ;  $p<.234$ ). This further supports the finding that all animals truly had acquired stable and consistent operant behaviour. Comparison of latency after stimulus onset to pre-stimulus leverpress behaviour clearly shows a difference ( $F=12.698$ ;  $p<.001$ ), which indicates that the CS-US association was established adequately. There were, however, no differences between the groups with respect to latency after stimulus onset ( $F=1.537$ ;  $p<.235$ ): The suppressive capacity of the stimulus was similar for both groups.

As can be seen in Figure 2 a remarkable difference between the groups with respect to post-stimulus leverpress behaviour ( $F=4.887$ ;  $p<.044$ ) was found, whereby the wakefulness group continued leverpressing stably, whereas the SWS group continued unstably (i.e. with several interruptions and increased inter-leverpress latencies).

## DISCUSSION

Present results show that during wakefulness as well as during SWS presentation of an initially neutral stimulus is processed to such an extent that, after excitatory conditioning to an US, the stimulus has considerable suppressive capacity on instrumental behaviour, despite the pre-exposure. Interestingly, the quality of the suppressive capacity differed markedly between the animals who got the pre-exposure of the tones during wakefulness and the animals who got the pre-exposure during SWS. The difference was not found in the latency to restart responding after stimulus onset but in the post-stimulus leverpress behaviour, which is the main outcome of this study.

This finding implies altered processing of the stimulus during SWS as compared to during wakefulness. The alterations are not likely to be simply quantitative in nature (e.g., a lower associative strength between CS and US for the SWS group), since the extensive literature on conditioned suppression states that quantitative differences should have expressed themselves in latency after stimulus onset. Rather, the difference in post-stimulus leverpress behaviour is caused by changes in the qualitative characteristics of the stimulus: It seems as if the SWS group has lost the causal temporal association between the CS and the US. The animal appears to be aware of the fact that the tone is associated with the presentation of a footshock, but lacks information about the temporal relation between the tone and the shock. This means that the stimulus does not predict the occurrence or absence of the tone adequately, it rather signals the animal for a 'uncontrollable' situation.

This suggestion is supported by the visual observations of the animals of the SWS group after stimulus onset. A striking and consistent behavioural pattern arises: at first, the animals completely stop pressing the lever after stimulus onset. Next, they occasionally huddle in a corner of the Skinner box chamber and freeze (see post-stimulus leverpress behaviour in Figure 2). In contrast to the animals of wakefulness group, the SWS animals do not respond to the off-set of the stimulus as if this was a 'safety signal' and the induced anxiety appears to be much more fierce. This observation shows striking similarities with observation described by Schwartz (1994.). He induced comparable behaviour by exposing rats to tones and shocks of which 50 % was explicitly paired and 50 % explicitly unpaired. Because of this procedure the animal is prevented to learn about the causal temporal relation between the tone and the shock.

Our animals received ten tone-no shock pairings (pre-exposure) as compared to three tone-shock pairings. To reach a situation comparable to the 50 % explicitly paired - 50 % explicitly unpaired condition, we therefore speculate that the stimulus presentations during SWS had a comparable perceptive value as 'explicitly unpaired' presentations but recall from this presentations is not as adequate as compared with the recall from the stimulus presentations during wakefulness. At present, two mechanisms are suggested in the literature to explain this altered stimulus proces-

sing: Firstly, the perception and concomitant evaluation of the tones may be less adequate during SWS. This was accounted for by the transfer-ratio concept of Coenen & Vendrik (1971), as the output of the thalamus to the cortex is diminished during SWS as compared to wakefulness. Secondly, several studies have suggested that the entrance of information to Long Term Memory during SWS is diminished as compared to during REM sleep or wakefulness (McDonald et al., 1975; Hars & Hennevin, 1987). The latter authors argue that during SWS associational learning cannot reactivate the whole memory trace, which leads to a "disorganized memory trace". These two mechanisms, be it in an additive or synergistical way, may have caused our animals in the SWS group to appreciate the pre-exposure tones as as informative with respect to predictive value for the shock as the explicitly unpaired presentations. Future studies may elaborate on the paradigm used in the present study to, obviously, further elucidate the role of the suggested mechanisms in altered information processing during sleep.

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