

Effects of environmental enrichments and olfactory stimuli on sleep pattern: an application of integrating information on behaviour and brain electrical activities

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Following differential experiences provided by enriched environmental conditions, several studies have shown significant changes in laboratory animals at morphological and behavioural levels [1, 2], but only few and not recent works evaluated effects of these conditions on sleep pattern [3, 4]. Besides, some data suggested the correlations between cortex, hippocampal electrical activities and olfaction [5].

The aim of this study was to identify possible modifications of the behavioural pattern and/or of the concurrent recorded electroencephalographic traces in rats as a consequence of different environmental conditions (i.e., enriched or not enriched) and in the presence of olfactory stimuli applying the concept of integrating acquisition and analysis of physiological parameters with the behaviour of laboratory animals. In this research we used an integrated system for the simultaneous and synchronous recording of the behaviour by video (by Noldus Information Technology) and physiological EEG and EMG signals by telemetry (by Data Science International), which was installed and validated in our laboratory.

Four male CD™ rats, approximately 7-8 weeks old, were selected for the surgical instrumentation with a telemetric transmitter (type TL10M3-F50-EEE, DSI) which allows the simultaneous recording of cortical and hippocampal (deep into the *dentate gyrus*) electroencephalograms and the electromyogram EMG (on the neck muscle) [6]. After approximately 45 days of post-surgery recovery, rats were singly housed and habituated for 5 days into the experimental PhenoTyper cages (by Noldus Information Technology). After this relatively short habituation period, a background registration session was conducted acquiring simultaneously and continuously for 24 hours the telemetric signals using the acquisition system Data Science International Dataquest ART Gold 4.01, and the general activity of animals by collection of videos using the MPEG4 Encoder and The Observer XT connected directly with the PhenoTyper cages. Three days after this background recording, two further sessions were conducted, in which 2 out of the 4 animals received a novel environmental enrichment (3 objects: tunnel, ball and rodent house) into their cages. The first recording's session was conducted immediately and the second three days after the novel environmental enrichment. Three days later, two additional sessions of recording were conducted, in which for 4 consecutive days at approximately 8.00 am all 4 animals

received an olfactory stimulus (3 drops of bergamot and 3 of lemon essential oil applied into the bedding material – common perfumes exhaled by disinfectants used for cleaning procedures) into their cages; the first recording's session was conducted immediately after the first occasion of the olfactory stimulus and the second on the fourth day of olfactory stimulus.

On each recording's occasion the telemetric signals and the videos were acquired simultaneously and continuously for 24 hours, starting at approximately 8.00 am and stopping at the same time (8.00 am) of the day after.

Telemetric EEGs, EMG waveforms and the activities of animals collected with our integrated telemetric and video system will be presented thus providing unprecedented insight into the correlations between behavioural and brain electrical activities following environmental and olfactory experiences.

All the experiments were carried out in accordance with Italian regulation governing animal welfare and protection and the European Directive 86/609/EEC, and according to internal GlaxoSmithKline Committee on Animal Research & Ethics (CARE) review.

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