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Development of a method of exposed characteristic points in activity pattern for rat behaviour classification

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A fast method for visual inspection and classification of massive locomotor activity data registered from laboratory rats is presented. Positions in the home cage of one hundred rats have been constantly recorded during 90 day period using photodiodes and beam crossing method with use of custom build system. Direct inspection and comparison of classic form of actograms did not bring information for fast and easy recognition of anomalies in daily behavioural cycle. A method of obtaining fast and easy to compare locomotor activity pattern is presented. The key point of proposed method is exposition of characteristic points in the activity diagram. About 9000 actograms were inspected and classified for investigation with use of ANOVA.

Key words: preclinical test, locomotor activity, actogram, behaviour recognition, rats.

Introduction

Subtle changes in behaviour of animal model are the scope of interest of psychotropic drug research [5,8]. These subtle changes in behaviour are repeated on daily basis and manifest themselves in small increase or decrease of activity within 5 minute before or after light-on or light-off stimulus. Automated locomotor activity registration methods are very useful in removing the subjectivity and bias out of measured behavioural parameters in rodents [2,7]. These studies must not be conducted in novel environments, but in ones that the animals are familiar with, such as their home cage [3].

Locomotor activity data can bring valuable information about even slight behaviour change upon drug influence. While whole daily distance or pattern of daily movement is a matter of biological rhythms, the reaction of organism upon stimulus is domain of behavioural research [9]. Behavioural reaction manifests as short time activity change as a reaction for light-on and light-off stimulus. Such changes can be detected

and statistically analysed of adequately large probe. In our case this probe was five groups of twenty animals each monitored simultaneously. Each of the group has slightly different breeding condition such as light-on phase duration or food composition. Subtle changes in activity level are difficult to analyse directly, by examining actogram presented in conventional way as shown in Figure 1. These changes can be clearly seen by use of statistical methods. Before application of automated analytic methods for behaviour change detection, the first step is filtration and selection of valid data. Data for investigation are taken at days with no artificially induced change of activity pattern of given rat. Example of invalid dataset due to personnel intervention at 1:30 p.m. in laboratory room is shown in Figure 2.

Figure 1. Example of conventional actogram.

Figure 2. Example of conventional actogram with visible effect of personnel intervention in the laboratory room at 1:30 p.m.

Material

One hundred of young male Wistar rats were constantly monitored in individual home cages during 90 days period. Data were collected during experiment conducted at the animal house of the Medical Research Center of Polish Academy of Sciences. The rats had unrestricted access to food and water. Temperature and humidity was kept at

constant level. Home cages have dimensions of 425x266x185mm. Position of every rat in its home cage was measured using photodiodes and beam crossing method and recorded in the database. The measuring beam grid consists of 8 by 14 infrared transmitter-receiver pairs with spacing of 28 mm. Each rat was identified by the number of its home cage.

Method

Initially, rat position in the cage was registered 8 times per second. After that, displacement is summated and divided by the period of quantization giving displacement in given quantization period. A sample of conventional actogram with quantized distances covered of given rat is presented in Figure 1. Such data presentation method is not efficient for the exposition of characteristic points within a whole day cycle and it is difficult to compare such sets of graphs in case there are thousands of them.

The problem was solved by conversion of data. Given the registered positions of the rat in the home cage, a distance of the rat movement can be precisely computed. The cumulative displacement forms an ascending curve, as it is displayed on the graph. By subtracting trend value from these cumulative displacements a new curve is formed shown in Figure 3. Graph of proper behaviour should have mostly a falling down slope in the day period and an ascending slope during the night. Such activity pattern exposes natural undisturbed behaviour of rats.

Figure 3. Proper plot of undisturbed pattern of locomotor behaviour.

Results

The characteristic points in the plot in Figure 3 are:

- light-on at 7 a.m. visible as slope change from ascending to descending,
- light-off at 8 p.m. can be seen as slope change from descending to ascending (Figure 3).

Influence of personnel intervention in laboratory compartment in some days, for animal weight measurement or food and water supply, can be recognized as ascending segment during light-on phase, and is similar for all rats monitored in given laboratory room. Effect of personnel intervention into laboratory compartment at 2 a.m. is illustrated in Figure 4.

Figure 4. Plot of pattern of locomotor behaviour with visible effect of personnel intervention into laboratory room at 2 p.m.

Data which should be excluded from later analysis concern the days with personnel intervention, and with evident behaviour abnormality induced by other reasons like illness or pain, etc. or by unknown factor. Example patterns of easy to distinguish unnatural, disturbed behaviour are shown in the Figure 5 and Figure 6. In the Figure 5 the rat was unnaturally active from 12 a.m. to 6:30 p.m. In the Figure 6, the rat was unnaturally active from 11 a.m. to 5 p.m.

Additional sources of inappropriate data can be sensor failures, power failures, animal deaths etc. We observed that low level of hysteresis in photoreceiver becomes a possible source of erratic data by effect of flickering of rat shadow. This can induce false readout as rat quickly moves between two neighbouring positions. The latter can cause false distance to be accumulated and we were able to filter this out by software mode.

Figure 5. Example of the pattern of unnatural, disturbed behaviour.

Figure 6. Sample pattern of unnatural, disturbed behaviour.

Conclusions

The use of characteristic points allowed us, to remove the inappropriate data easily and fast, from being analysed in later phase of data processing.

Initially, without any selection, dataset consists of 9000 diagrams of one rat per day. Finally, after careful examination, about 8600 datasets were selected for future study.

The proposed method provided a valuable help and it was impossible to prepare the software tool to perform adequate data filtration automatically.

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