Rats have a complex repertoire of social behaviors. In their natural habitat they live in groups but may share resources such as food and water. In laboratory situations in the presence of a receptive female, male rats rapidly develop agonistic behaviors towards unfamiliar conspecifics. As social behavior is an accessible “read-out system”, it is often used for studying brain-behavior relationships, e.g. as a model for anxiety. There is literature on the consequences of social isolation on behavior and physiology in rats. Nonetheless, the mechanisms underlying this apparent drive to keep in social contact have not been elucidated. Part of our research is focused on the behavioral and physiological characteristics of this phenomenon.

**Experimental approach**

A reliable method to recognize the effects of isolation is indispensable. The effects of isolation are best seen in a social interaction when the isolated animal demonstrates more social interest. The test cannot be performed in the home cage of the socially housed animal as familiarity with the cage is an important parameter affecting behavior. In a novel environment a variety of exploratory and social behaviors are displayed and an isolated animal is not easily distinguished from a socially housed animal. Only when social behaviors are continuously monitored by a human observer do differences appear. For regular testing, a more quantitative measure is required and therefore EthoVision is used. The coordinates of the center of gravity of each animal are continuously tracked. From these time series of coordinates the inter-individual distance and the direction of movement regarding the other animal are derived. At each time interval which is long enough for the animal to show directed movement (every 2 s), the direction and the length of the displacement of both animals is measured. The contribution of each animal (in terms of distance and direction) to a change in inter-individual distance is then assessed. In case of a decline of inter-individual distance, at least one of the animals is said to approach. With an increase of inter-individual distance, at least one of the animals is said to avoid. Of course, several combinations of approach and avoidance behavior of both animals are possible. Since approach and avoidance only make sense when the animals are not too far away from each other, directed movements are weighted based on the inter-individual distance; if more remote from each other, movements are more likely to be exploratory activities. Thus, directed movements such as approach and avoidance are very precisely and objectively calculated and expressed in cm, whereas the human observer counts the number of such behaviors. A close comparison of computer-collected data and data obtained by a human observer has shown similarities with respect to approach but differences with respect to avoidance. It appears that the human observer is inclined to identify movements away from each other as exploratory activities. When the human observer is asked why an increase in inter-individual distance is identified as exploratory activity rather than avoidance it appears that these differences are based on the continuous interpretation of the rat's presumed "intention". Quantitative measures as obtained by EthoVision are also more appropriate for statistical analysis.

**Results: opiates and social behavior**

Following this procedure we have been able to reliably demonstrate the effect of isolation. If an isolated and a socially housed animal were confronted with each other, the isolated animal was relatively more approaching than the socially housed animal. In addition, opiates were shown to affect the response to isolation. Isolated animals who had been treated chronically (two weeks) with morphine did not show any signs of isolation. Also local treatment with the ACTH(4-9) analog Org2766 into the central nucleus of the amygdala counteracted the isolation-induced enhancement of approach-like behavior. Prenatal treatment with morphine also influenced approach and avoidance behaviors in adulthood as assessed by EthoVision.
**Evaluation**

The power of an imaging system such as EthoVision lies in its ability to quantify movements in distance and direction in terms of cm and not merely frequencies. In patterns of movements new behavioral characteristics emerge, also when individual animals are tested in a novel environment. Effects of brain damage in the septo-hippocampal connection affected the exploratory activity when analyzed in this way. Time spent in the center of an open arena in the vicinity of an object increased. In fact, EthoVision has proven to be so useful in our laboratory that it is now applied for Morris maze behavior, exploratory activity and social interactions in brain-lesioned or drug-treated animals. It is most valuable in situations where more than one animal has to be observed and in long-lasting observation sessions. In the future, once detection of more subtle postures and movements becomes possible, behavioral scientists will have a more objective and more powerful tool to collect data and to attribute meaning to behavior.

**References**


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