BEHAVIORAL ANALYSIS OF PHARMACOLOGICALLY TREATED RODENTS IN AUTOMATED HOMECAGES

Thesis Book

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Introduction

Automated home-cages gained significant attention in the last decade as a fundamentally new way of studying behavior in rodents. They allow to either study various behavioral traits of disease models or make possible to follow the changes after pharmacological treatments. As rodents are kept in social environment and live by their own circadian rhythm, consequently they show more natural and less stressful responses to various tasks.

Spatial learning is a long-studied rodent behavior used in different tasks. By measuring it one can assume the learning abilities and memory capacity of a test subject. In traditional place preference learning task carried out in Y-maze, a group of mice can only be measured once. In automated home-cages where animals are kept for an extended period of time, a unique design is needed to overcome this limitation.

Many psychiatric disorders show dementia or cognitive impairment as a symptom, but it is most apparent in Alzheimer's Disease. A widely used pharmacologically induced rodent model of learning impairment is acute scopolamine treatment, which is an anti-cholinergic compound.

Autism spectrum disorder (ASD) is a condition affecting the development of the nervous system, leading to repetitive behaviors, impaired social interactions and reduced communication skills. Possible causes can be genetic or environmental.

Various behavioral assays have previously been used in rodents to measure autistic-like symptoms. These tests usually involve removing the rat from its home-cage, which can cause increased stress levels due to excessive handling by humans and being exposed to a new environment. Previously, many methods have focused on measuring a behavior trait of a single animal at a time. Rats, like humans, are a highly social species, so efforts were made in

this study to focus on group dynamics as opposed to individual rats, as social behavior and structure are affected in ASD. Observing rats throughout their natural circadian cycle and monitoring their undisturbed behavior patterns in a social environment was a priority and was possible with the use of automated home-cages. In this way, highly detailed information was collected. Valproate, or valproic acid (VPA), is an anticonvulsant medication with delayed developmental effects upon prenatal exposure. It also causes autistic-like traits - such as reduced social interaction, increased repetitive behaviors and anxiety - in the offspring of pregnant rats exposed to it. Treating pregnant rats with VPA and assessing the behavior of their offspring therefore creates a valuable model of ASD.

Objectives

Our main objective was to harness the power of huge amounts of data when analyzing rodents' behavior in IntelliCage. We aimed to establish a new standard methodology, accompanied by developing new statistical procedures to analyze appropriate parameters to follow behavioral changes. First, we aimed to establish a reward driven place preference learning paradigm in mice. We plan to apply an already well studied and documented mouse model of cognitive impairment, using an anticholinergic agent, scopolamine, to disturb the formation of long-term memory. To increase the challenge for the subjects, sequentially we plan to introduce reversal learning in a form of changing the rewarded corner regularly. We study not only the learning abilities of the mice during the process but the general activity, the nosepoking and the drinking behavior as well. In a separate study, we wanted to further characterize the rat VPA model, with a new equipment and with a new way of collecting and analyzing data, hoping we detect previously unknown

behaviors which could be utilized as potential new biomarkers. Our aim was to discover behavioral patterns when animals are kept in a social environment, relatively undisturbed while they are observed during their natural, undisturbed circadian cycle. We planned to establish a phenotype first with conventional methods used to study various symptoms associated with Autism Spectrum Disorder (ASD) of rats Using the phenotype data, we preselected a group of animals and designed a set of experiments using the IntelliCages. Communication is a pivotal part of social interaction and social cognition. As the latter domain is highly affected in ASD, it is an often-targeted area of study in ASD. However, adult animals' communication is hardly measurable. The process of forming hierarchy within a group of rats is shaped by social agonistic behaviors. It includes chasing, boxing, nipping, biting which behaviors are certainly the result of audio-visual communication between the animals. Hierarchy can be interpreted as a cumulative result of all these agonistic interactions. Studying the dynamics of the hierarchy, one could deduce the communicative skills of the autistic animals. We have run the experiment for an extended period with relatively minimal challenge. The last part of the study was an amplified competitive situation utilizing partial water deprivation, which urged the rats to establish a strict hierarchical structure within each group.

Methods

Vehicle and scopolamine treated groups of mice were used to study their place preference and reversal learning abilities. Mice were implanted with microchips prior to placing them into the IntelliCages. The cages were laid out in such a way that water was only available in the four corners which could

be visited by just one individual at a time. The corners were able to record the microchips of the rats to identify the subjects. A sequential training process began with an acclimation period allowing them to drink *ad lib*. Secondly, Mice had to perform a nosepoke to open a door which hides to bottle nips where they can drink for 7 seconds. In place preference learning task, only one corner was correct while the three remaining corners were incorrect. In the incorrect corners they also had the opportunity to drink for 7 seconds but also received an aversive stimulus, a rapid air-puff. Mice were evenly distributed in a way that 4 mice were appointed to the same corner In the final, most demanding task was a reversal learning paradigm, in which the position of the correct corner was rotated daily. In this task, they had access to perform the task for 3 hours per day, meaning a 21-hour long water deprivation before each session. Visits, nosepokes, licks and their durations were measured, various parameters calculated and compared.

Two groups of pregnant rats were injected with either vehiculum or VPA. F1 generation were than used for the subsequent experiments. A series of tasks were performed on the litters to assess autistic traits. A scoring system were applied to choose a group of rats with a relatively homogenous phenotype. Rats were then implanted with microchips prior placing them into separate IntelliCages, one containing VPA and the other normal rats. Similarly to the mouse design, the training process began with an acclimation, followed by nosepoke learning, side preference and reversal learning, and finally competition task. The water access became increasingly more and more limited. In the competition task, the rats were intentionally not equally distributed. All of them were appointed for only one bottle, to increase the chance of frequent social interactions. They had two access window each 2 hours long. Visits, nosepokes, licks and their durations were measured,

various secondary parameters, patterns and ratios were calculated and compared.

Results

Scopolamine increased locomotion of mice (measured by visit numbers) especially in the first 20 minutes. Scopolamine significantly increased nosepoke number/visit, and decreased visit durations. Nosepoke and lick durations were drastically decreased by scopolamine. The error rate of the scopolamine group in reversal learning task was significantly higher.

Prior to the main behavioral study, the VPA rats were tested for autistic-like behaviors. They demonstrated reduced rearing movements compared to the control rats, as well as reduced vocalization when separated from their mothers. The Von Frey test showed a significant elevation in sensitivity.
In the first 12 hours of the acclimation, VPA rats showed a significantly reduced initial exploration. VPA rats did not show impaired side preference or reversal learning. Decreased exploration remained characteristic for the VPA group throughout most of the experiment, except in the competition.
Analysis of the activity pattern revealed a circadian difference between the groups, meaning a two-hour delay compared to the control in the peak activity for the VPA group. Cry1, Per1, Arntl, Npas2, Clock and Mtnr1a gene expression levels did not show significant difference between the groups.
VPA rats showed a significantly increased lick number per hour, and lick duration per visit in all experimental phases. Blood serology data, aldosterone, anti-diuretic hormone and glucose levels did not differ significantly between the groups. In competition, the distribution of lick number per hour showed drastically different shape: control animals' data were fitted with a

logarithmic curve, while VPA rats distribution could be fitted with a “broken stick” model. Pielou’s evenness index showed no difference between the groups in acclimation and nosepoke learning phases. In side preference and reversal learning paradigms the VPA group showed a slight but significant increase compared to the control group, while in competition. VPA group showed a drastically higher and significant evenness compared to control group. Maximum per mean reentering visits were much more numerous when water was limited (side preference and reversal learning paradigms and competition) in the control group. Compared to this, when water was much less limited (acclimation and nosepoke learning), these reentering visits were significantly lower in controls. In VPA rats there were no difference between the reentering visit numbers when the water less or more limited.

Conclusions

Using automated home-cages, we established different rodent models of psychiatric disorders to study the underlying mechanisms. We analyzed huge amount of data gathered through a sophisticated, unique statistical approach. We also created new behavioral tests in two rodent species that allow us to investigate compounds to alleviate symptoms of various psychiatric disease models. These results will further validate our findings and will open the opportunity to test our own synthetized compounds as medium-throughput robust behavioral methods with high translational value. We led a pioneering work to harness the advantages of relatively stress-free environment for rodents and subjective bias-free data collection made possible by automated home-cages in pharmacological studies.

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Patents

Patent application pending (equal inventor share): György Lévay, Viktor Román, Kristóf Kelemen, Péter Pelsőczi File number: P1900442.
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