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Response to novelty in rats tested in isolation and in pairs: focus on exploration and play

The main goal of the study was to compare investigatory responses towards novelty in 20 Wistar rats divided into two experimental groups (solitary exploration vs. exploration in pairs). Additionally, relationship between novelty and social play/interaction was analyzed in the dyad group. Procedure involved placing animals in an experimental chamber during fifteen, six minute trials on successive days of the study. On the eleventh session a new object was introduced. The results are summarized within several behavioral categories. Investigatory responses of rats in dyad to novel object in familiar environment were not quantitatively different, than those of isolated animals. The animals from both groups responded to the novel object by focusing their exploratory activity on the source of new stimulation. Amount of social play and social exploration was influenced by the experimental manipulation with important sex differences present.

Keywords: *exploratory behavior, reaction to novelty, play fighting, social interaction, investigatory responses, rat*

Play and exploration are considered by ethologists and comparative psychologists as being closely related classes of behavior (Einon, 1983; Lorenz, 1982). Unfortunately the exact nature of that relationship still remains a mystery. The ultimate goal of studies on exploratory behavior is to recognize factors determining the ability of an organism to adequately react to environmental change. Recent theoretical proposal by Špinka, Newberry and Bekoff (2001) addresses the same goal in the realm of play behavior. Authors suggest that the function of play is to train an organism for the "unexpected" by increasing its ability to cope with environmental change. It contradicts the view, that the function of play is merely the development of species-typical adult behavior, e.g., agonistic, sexual or predatory behaviors. It is proposed, that play has a more global function. It is supposed to develop flexible emotional and kinematic responses to unexpected events. This process works in two inter-related ways. Playing results in creating a more diversified repertoire of behavior of an individual and secondly, increases emotional control in unexpected situations. Although authors quote some empirical evidence in support of their theory, they admit that many of specific hypothesis derived from it have not yet been verified. Providing more empirical evidence for them would be beneficial for studies of both exploratory and play behaviors, yet some preparation is needed beforehand.

The aim of the present experiment is to establish a ground base for studies on environmental factors affecting exploration and play. The species selected for such studies must display high levels of those behaviors. A test created for such a purpose must meet several criteria. The testing arena should enable animals to display a wide range of behaviors in a maximally naturalistic setting achievable in a laboratory. The methodology must provide a possibility to observe investigatory response to environmental change and play behavior simultaneously. In the following sections we present arguments for selecting common laboratory rats as a species for such analysis, arguments for using tests of exploratory behavior under low-stress conditions as the methodology backbone and the possible effect of environmental change on investigatory responses and play in such experimental setting.

In an evolutionary perspective both exploration and play seem to share an important feature. Intensity and complexity of both of them are positively correlated with behavioral plasticity and cognitive capabilities of an organism. The analysis of exploratory behavior can be traced back to simple, single cellular organisms, Pisula (2003a) provides extensive review of this subject, yet the most sophisticated and intensive exploration can be observed in primates

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(Gottlieb, 1992, in: Pisula, 2003a; Glickman and Sroges, 1966). Complex exploratory behavior is characteristic to animals living in a diverse environment, and thus in frequent need of adequate reaction to a variety of stimulus. High levels of play are also characteristic for animals functioning in a diverse, unpredictable environment. Animals of species that play also show the most intensive and sophisticated exploratory behavior (Glickman and Sroges, 1966). Play has been observed in a wide range of mammalian and avian species and is often thought of as limited to only those species (Bekoff and Byers, 1998; Fagen, 1981; Burghardt, 2005; MacLean, 1985).

Recent findings suggest playful like behavior in turtles (Burghardt, Ward and Rosccoe, 1996) and octopuses (Kuba, Meisel, Byrne, Griebel and Mather, 2003; Mather and Anderson, 1999). This has opened a discussion on phylogenic origins of play. Although detailed analysis of this argument is beyond the scope of this study, the most important part of this discussion is the question, whether we can treat different behaviors related to play as having a common function. Some authors argue that play is so diversified that each type should be analyzed individually. Others suggest that it is beneficial to treat play as a unitary phenomenon, and attempt to describe a common, basic function of play (eg. Špinka, et al., 2001), without negating the fact that many secondary, species specific functions of play may exist. The latter approach is assumed here.

The most common type of play exhibited across species is rough-and-tumble wrestling (Fagen, 1981). This is also the most common (82% of a sample of 166 papers dated 1984-1994) measure of play used in studies (Pellis and Pellis, 1998). Play fighting or rough-and-tumble wrestling is the primary form of play in laboratory rats (Pellis and Pellis, 1990) and these animals are often chosen as subjects in studies which examine neurological correlates of play (Vanderschuren, Niesnik and Van Ree, 1997). Rats also display an extensive repertoire of exploratory behaviors and their reaction to environmental change is well documented. There is some evidence that play and exploration are interrelated in these species. Pisula, Gonzalez-Szwacka and Rojek (2003) have found that juvenile play-fighting in rats correlated positively with two forms of adult exploratory behavior: touching and manipulating an object.

Studies of exploratory behavior under low-stress conditions usually involve placing a single animal in an enclosed start box, and giving it access to a large arena containing several objects. The animal is habituated to the experimental arena during first sessions on consecutive days and then a change in the environment is introduced, in the form of a novel object or rearrangement of existing objects (e.g., Pisula, 2004; Pisula, Stryjek and Nałecz-Tolak, 2006). The animal's activity in the arena on each day is then quantified. This type of test is considered the least constrained and the most ecologically valid measure of an animal's exploratory tendencies in a laboratory setting (Renner and Rosenzweig, 1986; Renner, 1987; 1990).

It must be taken into consideration, that the majority of experiments in this setting measure the behavior of a single rat. This means, putting the animal in short term isolation from the group. When measuring play fighting, an obvious change must take place with two rats tested simultaneously. There is only scarce empirical evidence on how that would affect exploratory behavior of rats. It can be hypothesized that the intensity of exploratory behavior will be higher in rats tested in dyads. This prediction coming from the fact that the presence of a conspecific decreases predatory risk (Roberts, 1996), which is a factor greatly influencing exploratory behavior intensity (Pisula, 2003b). Engaging in a solitary exploration of novel environment leads to increased vigilance and higher arousal levels than exploration with a group of conspecifics (Roberts, 1995). When the same animal is tested alone and in the presence of its peer group, it shows more intense exploratory behavior in the latter condition (Genaro and Shmidek, 1999).

On the other hand, some facts suggest that the proposed experimental setting might not be suitable for testing pairs of rats. When the introduction of a novel object takes place after an extended period of habituation, animals are already familiar with the arena. Those tested in dyads would possibly display considerable amounts of play fighting. Play fighting is a robust and vigorous behavior also associated with decreased attention (Špinka, et al., 2001). Animals engaged in social play might not notice a subtle and only slightly ethologically important change in the environment. That interpretation would be in conflict with the hypothesis, that play is undertaken only in a safe environment (Špinka, et al., 2001). According to that hypothesis rats should explore the environment in the beginning of each session before commencing play.

Taking this problem a step further, one of the most complex and interesting objects in the rats world is another rat. When choosing between exploring unfamiliar space/object and unfamiliar conspecific, rats choose the other (Latane and Glass, 1968). It might be that rats tested in pairs will direct most of their time budget in the experimental chamber towards interaction with a familiar conspecific, ignoring subtle, non aversive environmental change.

Creating low-stress conditions for measurement of play behavior is even more important than creating such conditions for the measurement of exploration. Play is rarely seen in situations involving subjective uncertainty or fear (Fagen, 1981) and easily suppressed when aversive changes appear in the environment. Risks associated with predation greatly affect playfulness. Ecologically valid stimuli like acute exposure to predator odor can lead to a powerful, long lasting suppression of play behavior (Siviy, Harrison and McGregor, 2006). Play is almost totally suppressed, when rats encounter tufts of cat fur in an area providing them daily opportunities to play (Panksepp, 1998). Anxiety-provoking stimuli that lack ecological validity like sudden changes to the environment can disrupt a previously ongoing bout of play (Siviy and Baliko, 2000) for a short period of time. It returns to the baseline level when the environment returns to the previous state. Levels of social interaction (social play and nonplay behaviors) are more sensitive to aversive changes (bright light, unfamiliar arena) than exploratory behavior or locomotor activity (File and Hyde, 1978). In the realm of social behavior, social play is the most sensitive to such changes. Exposing rats held in dim light conditions to bright light suppresses play, but not other social interactions (Knutson, Burgdorf and Panksepp, 1998). Those findings suggest that play is the most sensitive to changes in the environment, followed by social and object exploration.

One of the functions of exploratory behavior is to identify dangerous aspects of an environment (Špinka, et al., 2001). Once the environment is assessed as safe, play can commence (Špinka, et al., 2001). When rats are tested in an unfamiliar environment, they will explore the cage before initiating play (Vanderschuren, Niesink, Spruijt and Van Ree, 1995). This sensitivity of play to intensive stimulus could provide studies on exploration with a new, important indicator of emotional state. One can assume that when in contact with high levels of novelty, observed levels of play would not return to baseline levels until the organism has assimilated this change or assessed the threat value of the novel event as minimal.

It is worth noting the classical view of transition from exploration to play (Hughes, 1983; Hutt, 1966). It states that the series of behaviors preceding object-play in children begins with behaviors focused on learning to manipulate an object, succeeded by more diverse exploration, habituation and culminating with play. According to Hutt (1966) a child explores an object by asking the question, "What is this object?" and, when transforming it's behavior to play, it asks, "What can I do with this object?". It's obvious that this transition cannot be directly generalized on nonhuman animals. The questions related to play should rather sound like, "what could happen if this object was dangerous?" in case of object play, or "what could happen if this environment was dangerous?" in case of locomotor or social play.

In a stable environment in which un-threatening novelty occurs, an animal directs its exploratory behavior towards the source of novelty (Renner and Seltzer, 1991). According to Pisula (2003b) moderate levels of novelty evoke highest levels of exploratory behavior. There is some evidence that this effect also applies to different types of play.

When in contact with a moderately dangerous prey, cats tended to play with it before, after, or instead of, killing it (Biben, 1979). When object play in adult cats is terminated, due to habituation to sensory characteristics of the object, introducing a novel object after a short delay results in recurrence of play at high intensity (Hall, Bradshaw and Robinson, 2002) Horses observed in a novel environment exhibit locomotive play (Stamps, 1995). Novelty induces play even when an animal does not play "with" the novel object, but in the arena containing that object. Peak of play behavior in calves is observed after providing them with novel stimulation (Jensen, Vestergaard and Krohn, 1998). In a study involving piglets, introducing a novel object into familiar environment resulted in increased investigatory behavior during the first minutes of each session. This was later replaced with a significant increase in play (i.e., scampering and sparring) and decline of exploration (Wood-Gush and Vestergaard, 1991). Putting a novel, inanimate object in the home cage was a procedure used to stimulate play fighting behavior in rats in a study by Pisula, Gonzalez-Szwacka and Rojek (2003). Darwish, Koranyi, Nyakas and Almeida (2001) report that placing inanimate objects in a home cage of group housed rats resulted in appearance of social play, with males displaying this behavior at higher frequency than females.

One of the specific hypothesis derived from the Training for the Unexpected theory (Špinka, et al., 2001) states that moderate levels of novelty and unpredictability should increase the levels of play. This is because novel situation gives an opportunity to generate creative responses to the environment, which might then be more accessible in time of emergency. This view is in contrast with one of the predictions based on the "surplus resources model" (Burghardt, 1999), which explains play behavior referring to extended juvenile period that involves parental care and surplus resources. According to Burhardt's model, animals should display increased play behavior when the environment provides low amounts of stimulation.

In relation to present experiment, one may conclude, when animals are habituating to the experimental chamber, the play behavior should be suppressed, as it is only displayed in environment assessed as safe. Moderate amount of novelty should evoke play, as predicted by the Training for the Unexpected theory. Thus introducing a novel object to the arena should lead to an increase in play behavior.

In summary, the present experiment validates the following hypothesis. Rats tested in dyads, versus those tested in isolation, will present less anxiety-related behavior before exploring a novel arena. On the operational level this means shorter latencies before entering the novel experimental chamber in the first experimental sessions. Rats tested in dyads will present more intensive investigatory behavior towards a novel object introduced after a period of habituation to the environment, than rats tested in isolation.

For the play behavior, observed only in the dyad group. One can predict that when in contact with high amounts of novelty, like entering a novel arena, exploration, assessment

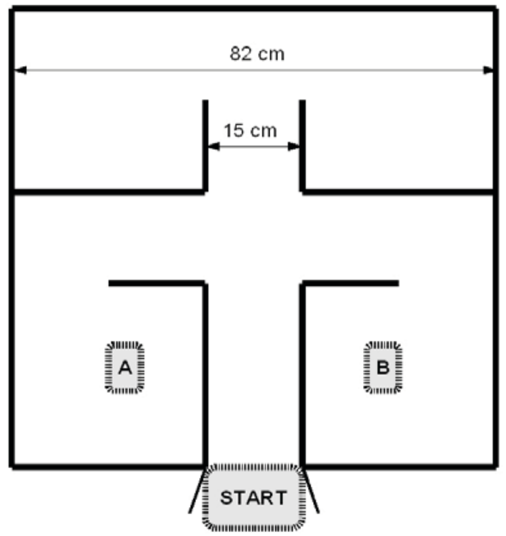

Figure 1. Experimental chamber. The container used to place the animal in the chamber was placed in the start zone. Sections A and B contained objects made of red LEGO blocks.

of risk and reduction of uncertainty is necessary, before play can commence. On the operational level, it means that the amount of play behavior displayed during the first two sessions will be lower than during the middle sessions (before the introduction of novel object).

Method

Subjects

The subjects were 20 (ten males and ten females) naive outbred Wistar rats. They were housed in transparent plastic cages; three or four rats per cage in temperature controlled (22 C) rooms with food and water available ad lib. Rats were about 90 days of age at the beginning of the experiment. This is a period of the most intensive exploratory activities in rats (Pisula, 1997; Renner, Bennet and White, 1992).

Apparatus

The experimental chamber (82x82x20cm) was made of wood, painted white and covered with Plexiglas. Inner walls were made from the same material. It was illuminated with red light of low intensity. Figure 1 presents a diagram of the apparatus.

Procedure

Before the beginning of the experiment a procedure of habituation to the transportation container took place. Each animal was placed in the container for one minute daily during seven days before the main experiment. Rats were then divided into two experimental conditions. Eight rats (four male and four female) were tested in isolation (group E1) and twelve rats (six male and six female) were assigned to six, unisex, dyads (group E2).

Every day, from the beginning of the experiment onward, each animal was brought into the experimental room in the transportation container. Container with one rat, in E1 condition, or a pair of rats, in the E2 condition, was placed in the start zone of the experimental chamber. After opening the container the experimenter left the room. The duration of each trial was six minutes. After each trial, the floor of the chamber was wiped with a moist paper towel.

Experimental procedure is presented in Table 1. It involved 15 sessions on consecutive days. The first ten days were the habituation sessions, and in the 11th session, novelty was introduced. The object in place B was replaced with a novel, differently shaped object, made from the same material. No other changes were made till the end of experiment on the 15th session.

Trials on sessions numbered 1, 2, 5, 10, 11, 12, 14 and 15 were video recorded. The camera did not generate any noise (also in the ultrasonic frequency range) that could affect the animals' behavior. The analysis of the video tape recordings was performed afterwards, with each six minute session divided into two, three minute, intervals.

The following behavioral activities were measured in both experimental conditions: (a) entry latency, defined as total time before moving from the familiar transportation container into the experimental chamber at the beginning of each trial; (b) object exploration, defined as touching, sniffing and biting objects. The following activities were measured only in the E2 condition: (a) social exploration, defined as sniffing or licking any part of the body of test partner; (b) social play, defined as pinning, (one of the animals lying with its dorsal surface on the floor with the other animal standing over it) boxing/wrestling and chasing (rapid movement in the direction of / or pursuing the test partner).

When rats are tested in dyads behavior of one influences the other, so they should be treated as a unit (File, Seth, 2003). In this experiment the behavior of individual animals was assessed, but for later analysis scores from the E2 condition were pooled together. For comparative purpose results of eight rats tested in the E1 condition were randomly joined to form four pairs.

Table 1
Course of the Experiment.

Session	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Recording	X	X			X					X	X	X		X	X
Procedure for both groups	Habituation period										New object in section B	Post manipulation period			

Results

The experimental design was based upon repeated measures and the main focus was on the differences between solitary and group condition in habituation to the environment and reaction to the experimental manipulation, introducing novel object. SPSS 12.0 for Windows was used for the analysis. Two-way repeated measures ANOVAs were made to assess the effect of group, sex and session on entry latency and object exploration. For the variables present only in the E2 condition additional repeated measures ANOVAs were run to assess the session and sex effect on social exploration and social play. In each analysis different set of sessions was used, just as presented on figures depicting results. The selection was made to focus on a period most relevant to each behavioral activity. This means the habituation sessions in case of entry latency, pre and post manipulation sessions in case of social and object exploration and the whole course of the experiment in case of social play. The results are summarized within those behavioral categories.

Entry latency

For entry latency, a significant interaction effect between group and session was observed, $F(5,25) = 3,52$; $p<0,05$; partial eta squared $= 0,41$. During first session entry latency was lower in E2 group, $t(7) = 2,40$; $p < 0,05$, and decreased

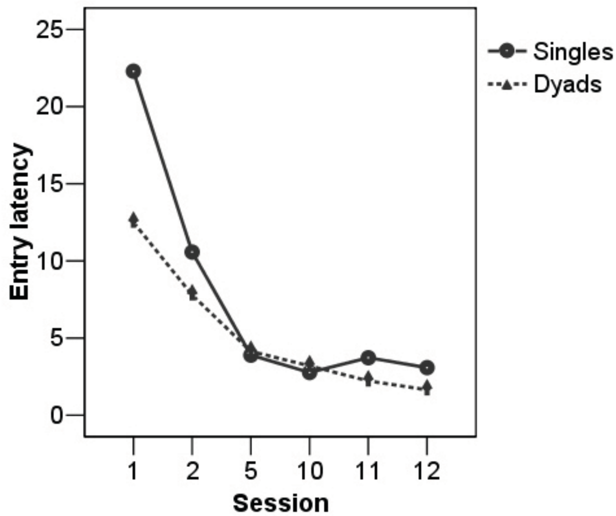

Figure 2. Average time (in seconds) spent by rats in a familiar compartment before entering into the experimental chamber during the habituation sessions (1,2,5,10), manipulation session (11) and post manipulation session (12).

from 1st to 5th session in both groups, as shown in Figure 2. It must be noted however, that the results of this analysis should be carefully interpreted. The Mauchly's sphericity test was not significant, but reached a low level ($p = 0,06$). During the first two trials higher within group variability of E1 vs E2 condition was noted. By the 5th session within group variability and mean entry latencies reached similar level for E1 and E2 conditions. The mean and standard

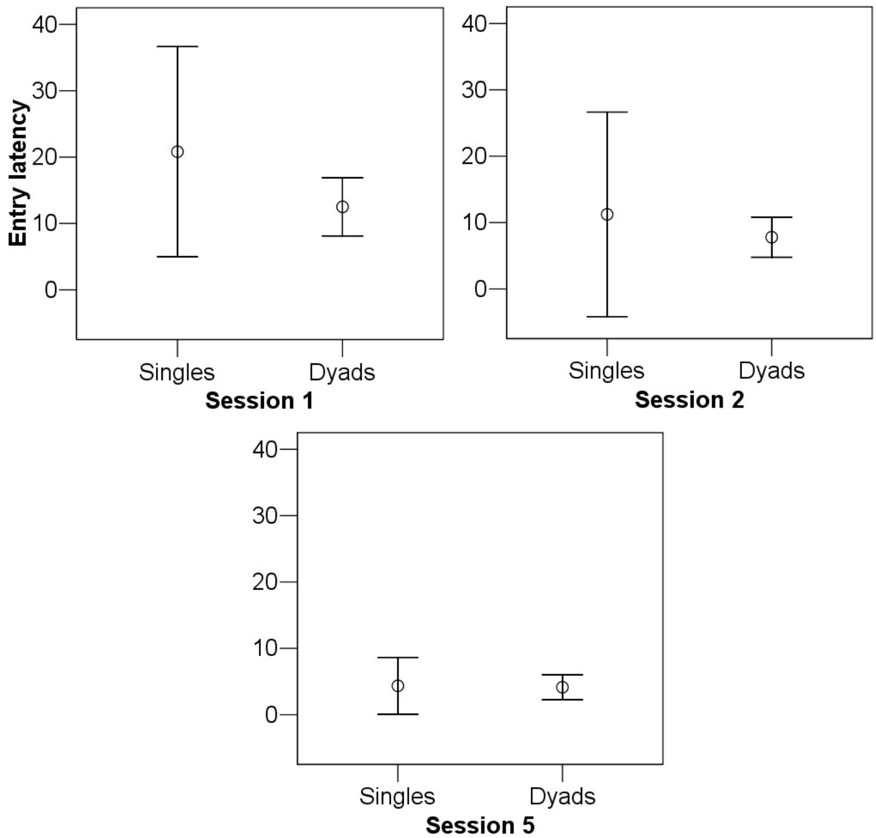

Figure 3. Average entry latencies (in seconds) for the E1 (singles) and E2 (dyads) groups. Vertical bars denote 95% confidence interval.

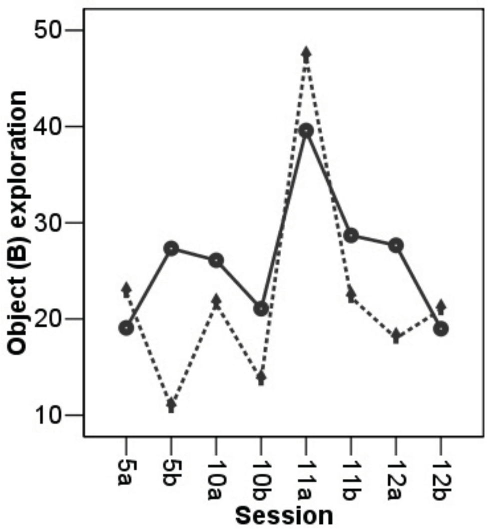

Figure 4. Average time (in seconds) of interaction with object in position B during the habituation sessions (5,10), manipulation session, introducing novel object in this position (11) and post manipulation session (12) each divided into first 3 min (a) and second 3 min interval (b).

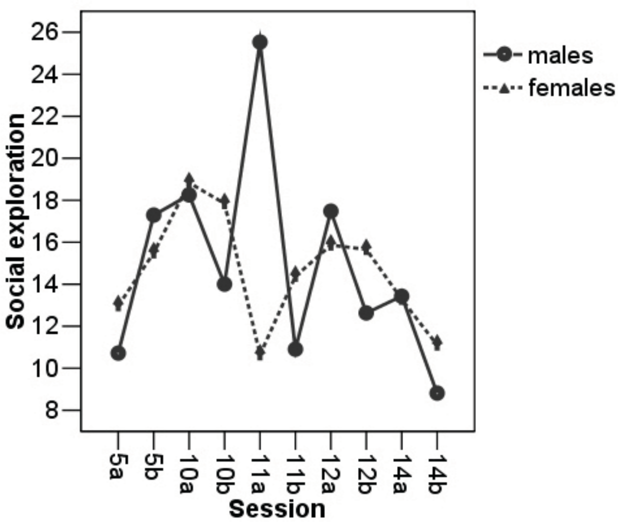

Figure 5. Average time (in seconds) of social exploratory behavior during the habituation sessions (5,10), manipulation sessions (11) and post manipulation sessions (12,14) each divided into first three min (a) and second three min interval (b). Difference between males and females reaches significance (p<0,05) only in the first interval of the 11th session.

deviation values were respectively: session 1 M_{E1} = 20,82 ; SD_{E1} = 6,36; M_{E2} = 12,50 ; SD_{E2} = 4,17; session 2 M_{E1} = 11,24 ; SD_{E1} = 6,20; M_{E2} = 7,79 ; SD_{E2} = 2,87; session 5 M_{E1} = 4,35 ; SD_{E1} = 1,73; M_{E2} = 4,14 ; SD_{E2} = 1,81. This result is shown in Figure 3.

Object exploration

As shown in Figure 4, the introduction of a novel object in the 11th session resulted in a temporary increase of exploratory behavior, $F(7,42) = 5,39$; $p<0,001$; partial eta squared = 0,47. The average time spent in interaction with object in position B was higher on the first interval of the 11 session in both groups, in comparison with baseline

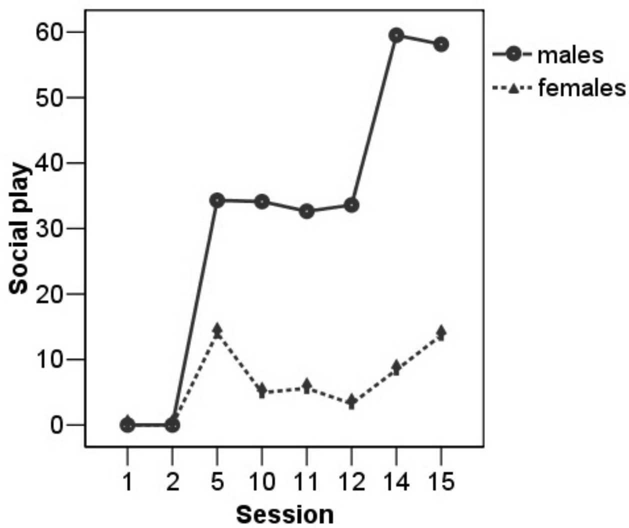

Figure 6. Average time (in seconds) of social play during the habituation sessions (1,2,5,10), manipulation session (11) and post manipulation sessions (12,14,15).

level. There were no differences in average time spent in interaction with object in position A.

Social exploration

There were no statistically significant effects of sex and trial on the levels of social exploratory behavior. But, a more detailed analysis revealed a result that can be interpreted in terms of the reaction to the environmental change introduced in the 11th session. In the first interval of the 11th session there is a significant difference between the sexes, with males displaying more social exploratory behavior, $t(4) = 3,81$; $p < 0,05$, than females. This result is shown in Figure 5.

Social play

Social play behavior revealed profound sex differences across the trials, $F(7,28) = 3,43$; $p < 0,01$; partial eta squared = 0,46. Rats began to display social play on trial 5, with males showing a tendency towards being more active. Males further increased amount of social play and they were significantly more active than females on sessions 14, $t(4) = 8,67$; $p<0,001$ and 15, $t(4) = 2,79$; $p<0,05$. This result is shown in Figure 6.

Discussion

The most important question, to which an answer was sought, was whether low-stress exploratory setting will be appropriate to measure rat dyads. The results are promising. The hypothesis which concerned disparities in exploratory behavior between the isolated rats and dyads has been partly confirmed.

The results suggest that the animals from the dyad group began the exploration of the novel environment faster than those from the isolated group. Additionally, it seems that the presence of a conspecific resulted in minimizing the individual differences between animals in respect to entry latency when in contact with novel environment. Granted that individual differences when in contact with a novel environment are mainly due to emotional reactivity levels (Pisula, 2004), one may consider animals showing below average (relative to their group) entry latencies as having lower emotional reactivity, and those showing above mean entry latencies as having higher emotional reactivity. It seems that in case of animals tested in dyads, those of pair, with lower emotional reactivity did not benefit greatly from the presence of the other subject in the familiar transportation container. That is, their entry latency is on a comparable level with the lowest scores of isolated animals. The observed difference is probably due to decreased entry latency of more emotionally reactive rats tested in dyads. Assuming that the less reactive subject of the dyad enters the novel environment first, it changes the situation for the other rat – the novel environment now "contains" a conspecific and might be assessed as less threatening.

Rats directed their investigatory behavior towards the novel object introduced after a period of habituation to the environment. Intensity of that reaction was similar in both groups. It means that rats tested in dyads, despite the presence of a conspecific and intensive play fighting, scanned the experimental chamber during each session and reacted to changes. Lack of differences in investigatory responses towards the novel object suggests that the presence of a conspecific influences exploratory behavior only when it's confounded with moderate or high emotional arousal. In the low stress situation, introduction of the novel object into familiar environment seems not emotogenic.

The second area of investigation concerned interaction between novelty and play behavior. In this aspect profound sex differences were observed. In this study the age of rats was chosen, as to maximize their exploratory behavior. Peak of exploratory behavior takes place about 40 days after a peak of play behavior. In this experiment males displayed more play fighting. This is in accordance with the notion that dimorphism of play increases with age (Špinka, et al., 2001). In the following studies, using younger subjects must be taken into consideration.

It is important to note that although males displayed more play fighting after a period of habituation to the environment, no sex differences were observed during the first sessions, as play fighting was absent in both males and females. It suggests that regulation of play in contact with novel environment is not sex specific. Rats played only after a period of exploration and familiarization with the environment. This result supports the view that animals play only in an environment assessed as safe (Špinka, et al., 2001).

Introduction of a novel object into the environment did not produce any immediate effects. Levels of play were not suppressed, as might be the case if the introduced novelty was assessed as threatening. It was also not elevated, as would be predicted by the Training for the Unexpected model (Špinka, et al., 2001). The only possible effect of the introduction of novel object on play could be the increase of play observed in males on the last two sessions. Unfortunately, due to the lack of comparison with a group not subjected to environmental manipulation, it's not clear, what is the cause of this effect.

Present study does not provide the sufficient data that would enable to state whether environmental change excites play. It is however possible to state that play is sensitive to environmental factors, as it's not displayed during habituation to environment. One interesting, and unfortunately not quantified observation from the present study, is that transferring rats to the experimental chamber elicited play. During the experiment, before each trial rats rarely played in their home cages, but from the 5th session onwards, each pair played in the experimental chamber.

An additional measure of behavior used, was social exploration. Obtained results confirm that in rats, a distinction can be made between forms of social behavior related and unrelated to play (Panksepp, Beatty, 1980; Vanderschuren, Niesnik, Spruijt, Van Ree, 1995). Social exploration was present during the first sessions, unlike social play. Conversely to play, the average level of social exploration was not affected by sex. There was however a single moment in which males and females differed significantly in respect to that measure. It was the first three minutes of the session in which the novel object was introduced. Males more intensively re-explored a familiar conspecific than females. It might mean that the general increase of exploratory activity evoked by the novel object was directed towards investigation of a conspecific. This might suggest a sex difference in respect to a reaction to novelty, which would be in accordance with previous studies. Hughes (1999) and Pisula and Siegel (2005) report that males are more sensitive to the occurrence of a novel object within a familiar environment.

Obtained results suggest that studying reaction to various types of novelty in rat dyads under low stress setting is well suited for determining environmental factors affecting play in rats, and thus verifying hypothesis from the Training for the Unexpected model (Špinka, et al., 2001). Investigatory responses of rats in dyad to novel objects in familiar environment are not quantitatively different, than those of isolated animals. Lack of play during the period of familiarization to the experimental chamber suggests that it is possible, using this setting, to suppress play without suppressing exploratory behavior. This enables investigation of the effect of novelty of varying complexity and intensity on play and exploratory behavior. It would in turn assist in establishing factors affecting reaction of an organism to environmental change.

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