Investigating the effect of risky play on cognitive performance and anxiety in rats

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Abstract

Risk-taking is an important component of play, through which children learn risk assessment, test and develop motor skills, and learn how to master challenging situations. Additionally, it has been suggested that exposure to risk alleviates anxiety. Unfortunately, little empirical research has been done to explore the contribution of risks during play for adaptive development. The aim of this study was to investigate the effect of risky play during the juvenile phase on cognitive performance and anxiety in adulthood. Rats were chosen as an animal model, since rats are playful animals and their play behaviour is well characterized.

A social play deprivation (SPD)-rearing method and a risky play cage were used to create four experimental groups: socially housed with and without 30 minutes of risky play twice a day (CTLR and CTL); and SPD-reared with and without risky play (SPDR and SPD). The probabilistic reversal learning (PRL) task, response inhibition (RI) task and 5-choice-serial-reaction--time-task (5CSRTT) were used to asses cognitive performance. The elevated plus maze (EPM) and large open field (lOF) were used to investigate anxiety, while a smaller open field evaluated locomotion.

The PRL task showed that CTLR and SPDR rats retrieved more rewards and displayed more win-stay behaviour than CTL rats. Additionally, introducing the variable ITI challenge did not affect omissions and correct responses in risky play rats, while performance in both low risk groups worsened during this challenge. While all groups displayed similar behaviour in the lOF, CTLR rats stayed significantly longer in the closed arms than both low risk groups. These findings indicate that risky play results in greater cognitive flexibility and altered anxiety-like behaviour in socially housed rats. Although performance of SPDR an CTLR rats were similar, no significant differences between SPD and SPDR rats were found. This could suggest that risky play is less effective in improving cognition in SPD-reared rats.

Statement of significance

Risk-taking during play has the potential to be beneficial for both cognitive development and attenuating anxiety. However, little empirical research has been done to explore the contribution of risks during play on cognition and anxiety-like behaviour. In this study, we addressed this problem by using rats and subjecting them to one of four experimental groups. To create these groups, both a social play deprivation-rearing method and a risky play cage were used. Multiple tasks were used to assess various facets of cognition and anxiety. We found that risky play opportunities lead to increased anxiety-like behaviour in socially housed rats, but not in social play deprivation-reared individuals. Additionally, we found that both risky play groups displayed altered cognitive flexibility.

Introduction

Play is an intrinsic rewarding behaviour that is commonly practiced amongst humans and most mammalian species (Brussoni et al., 2012; Gray, 2017). It creates a save environment in which an individual is able to experiment with their behaviour and to develop and practice motor, social, cognitive and emotional skills (Brussoni et al., 2012; Gray, 2017; Nijhof et al., 2018; Sgro & Mychasiuk, 2020). Play often entails risky conditions in which an individual can experience physical pain or rejection. Previous studies have indicated that through risk-taking, children learn risk assessment, test and develop motor skills, and learn how to master risky situations (Brussoni et al., 2012; Hansen Sandseter, 2007; Lavrysen et al., 2017; Orestes, 2015).

For example, Lavrysen et al. (2017) tested risk perception and competence in four and six year old children through a risk perception test, questionnaire and behavioural observations. Two classes were provided with risky-play activities, while two age-controlled classes received the regular curriculum over a period of three months. Children from the experimental group showed a larger improvement in risk perception and competence after the three month period compared to the control groups. In addition to improving risk assessment, risky play might also reduce anxiety through exposure to fear inducing stimuli (Allen & Rapee, 2005; Sandseter & Kennair, 2011). Unfortunately, little empirical research has been done to explore the contribution of risks in play for adaptive development. This is important as opportunities for children to experience outdoor free play have steadily declined (Brussoni et al., 2012; Clements, 2004; Gray, 2017; Karsten, 2005; Tandon et al., 2012). Acquiring more knowledge on the value of risky play, could help us improve mental development of both humans and animals.

Since animal research allows for more experimental control (Nijhof et al., 2018), animal models are often used to investigate the importance of play behaviour. As rats are playful animals and their play behaviour has been characterized well, they are most often used for play behaviour studies (Achterberg et al., 2014; Trezza et al., 2010; Vanderschuren & Trezza, 2014). Until now, mainly social isolation methods were used to asses the developmental impact of play. These studies show deficits in cognitive and social skills and impaired emotional regulation (Burke et al., 2017; Pellis et al., 2010; Sgro & Mychasiuk, 2020). Baarendse et al. (2013) looked at the influence of social isolation on impulsivity in rats by using a 5-choice-serial-reaction-time-task (5CSRTT). The socially isolated group made significantly more premature responses when test parameters were altered, which indicated an interference with impulse control. Additionally, Schrijver et al. (2004) showed that social isolation resulted in decreased cognitive flexibility. Rats that were socially isolated after weaning needed significantly more time to learn reversals in a two-choice discrimination task, which was explained by a selective impairment to reverse a previously established stimulus-response association. Next to cognitive impairments, social isolation during the juvenile phase increases anxiety, which has been shown by Arakawa (2003) with an open field test. Taken together, these findings suggest that play is important for an individual's development.

Typical laboratory settings for play research are limited in the actual risks animals may encounter. Sandseter (2007) described different categories of risky play: play with great heights; play with high speed; play with harmful and dangerous elements; and rough-and-tumble play. Apart from rough-and-tumble play and play with high speed, the other categories of risky play are not possible in the way laboratory rats are currently housed. Therefore, we developed a risky play cage to determine the effects of risky play on cognitive performance and anxiety-like behaviour. In addition, a social play deprivation-rearing method was used to create four rearing conditions that reflect a continuum of play behaviour ranging from no social play to a combination of social and risky play. Different cognitive processes, such as attention, processing speed and impulse control, were assessed with a probabilistic reversal learning task (PRL), responses inhibition (RI) task and a 5CSRTT. A large open field (lOF) and an elevated plus maze (EPM) were used to examine anxiety and a small open field (sOF) to evaluate locomotion.

Materials and Methods

Animals and housing conditions

All experiments were approved by the Animal Ethics Committee of the Utrecht University and the Dutch Central Animal Testing Committee. They were conducted in agreement with Dutch laws (Wet op de Dierproeven, 1996; Herziene Wet op de Dierproeven, 2014) and European regulations (Guideline 86/609/EEC; Directive 2010/63/EU). Three batches of 48 male Lister Hooded rats were obtained from Charles River (Germany). Batch one consisted of 52 rats instead of 48, because of a delivery mistake. They arrived on postnatal day (P)14 in litters of eight with nursing mothers. All rats were subject to a reversed 12:12h light-dark cycle with ad libitum access to water and food. All experiments were conducted during the active phase of the animals (9:00 - 17:00). One week before the start of behavioural testing, rats were subjected to food-restriction and were maintained at 4,5 grams of chow per 100 grams at 90% of their bodyweight for the duration of behavioral testing. Bodyweight was weekly measured and did not show significant differences in bodyweight between batches.

Social play deprivation and risky play

Rats were weaned on P21 and were either subjected to the control (CTL), social play deprivation (SPD), risky control (CTLR) or risky social play deprivation (SPDR) group. CTL rats were housed together in pairs during the whole experiment. SPD rats were pair-housed with a rat from the same mother. During P21 to P42, a transparent Plexiglas divider containing small holes was placed in the middle of the home cage of SPD rats creating two separate but identical compartments. SPD rats were therefore able to receive visual, olfactory and auditive cues from one another, but were unable to physically interact with each other. The Ple-

xiglas divider was removed on P42 and SPD rats were housed in pairs for the remainder of the experiment. CTLR and SPDR were housed under the same conditions as the CTL and SPD groups, but were transferred to a “risky play cage” twice a day for 30 minutes during the deprivation period (P21 – P42).

The risky play cage measured 149 x 36 x 40 cm (H x W x D) and contained multiple ladders, plateaus and other objects to interact with (See Figure 1). CTLR rats were placed together in the risky play cage, while SPDR rats played by themselves. All rats were housed in pairs until early adulthood (10 weeks of age) after which experimentation commenced.

Behavioural tests

Rats were provided with 30 sucrose pellets (45mg, BioServ) in their home cage before their first exposure to the operant conditioning chamber to reduce potential food neophobia. Rats were weighed and handled at least once a week throughout the course of the experiment. The three batches of rats were divided over the different tasks, to ensure that age at the start of behavioural experimentation were similar for each task. The first batch of rats executed the PRL task, the second batch the RI task and the sOF, while the third batch performed the 5CSRTT, EPM, lOF and sOF. The apparatus of every tests is shown in Figure 2. Two rats from batch three were taken out of the study during the 5CSRTT training phase, because they were suspected of having epilepsy. This could however not be confirmed by pathologic examination.

Probabilistic Reversal Learning

Apparatus: Behavioural testing was conducted in operant conditioning chambers (Med Associates, USA, Product: MED-008-D1) enclosed in sound-attenuating cubicles equipped with a ventilation fan. Two retractable levers were located on either side of a central food magazine into which sugar pellets could be delivered via a dispenser. A LED cue light was located above each retractable lever. A white house light was mounted in the top-center of the wall opposite to the levers. Online control of the apparatus and data collection was performed using MED-PC software (Med Associates, version 4.2.1.58).

Pre-training: Rats were first habituated to the operant chamber for 30 minutes in which the house light was illuminated. 50 sucrose rewards (45mg, BioServ) were randomly delivered into the magazine with an average intertrial interval (ITI) of 15 seconds between reward deliveries. On the subsequent days, the rats were trained for 30 minutes under a Fixed-Ratio 1 (FR1) schedule of reinforcement for a minimum of two consecutive sessions. The session started with the illumination of the house light and insertion of both levers, which remained inserted for the remainder of the session. A lever press on one of the levers was reinforced

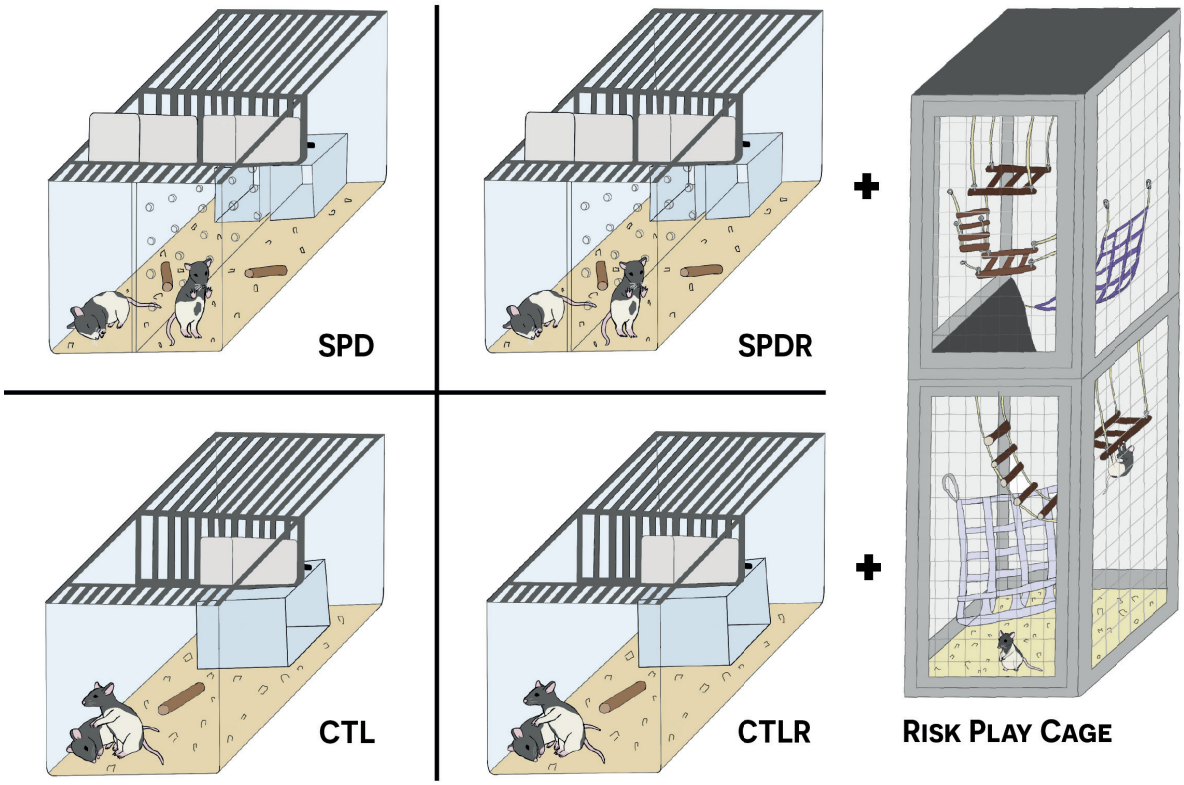


Figure 1: Social play deprivation (SPD)-rearing and a risky play cage were used to create four experimental groups. SPD and SPDR rats were housed with a litter mate, but with a perforated divider separating them during p21-42. CTL and CTLR rats were pair housed during the same period. SPDR and CTLR rats could play in the risky play cage twice a day for 30 minutes. After p42, all animals were pair housed and left in their home cage until behavioural experimentation

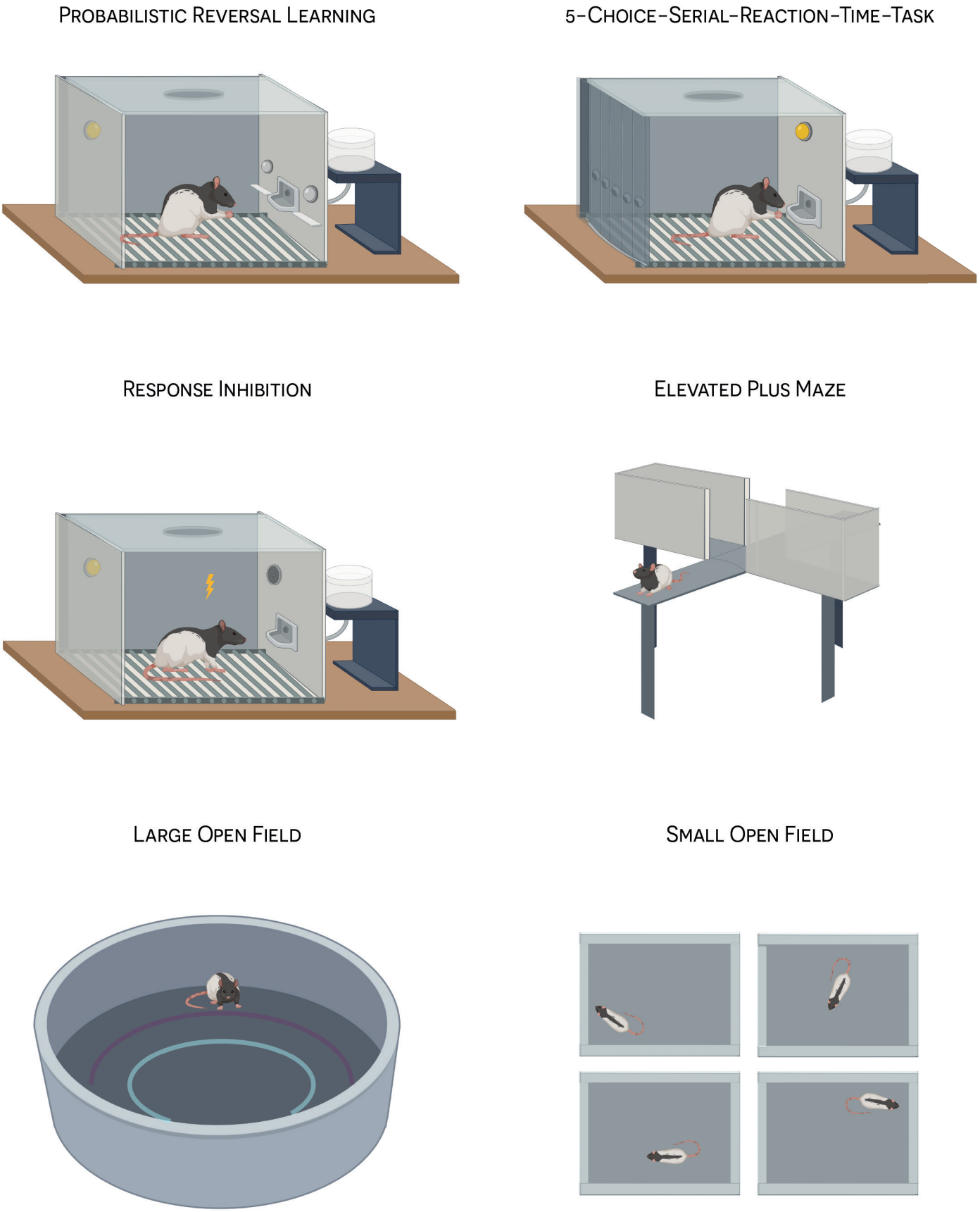


Figure 2: Multiple behavioural tests were used to asses the effect of social play deprivation and risky play on cogniton and anxiety-like behaviour. This figure shows the set-up of each test used in this study. The Probabilistic Reversal Learning task, 5-Choice-Serial-Reacti-on-Time-Task and Response Inhibition task were used to assess cognitive function. Anxiety was evaluated with the Elevated Plus Maze and Large Open Field. Potential locomotion differences were examined with the Small Open Field.

with a delivery of a sucrose pellet into the magazine. Rats could press the levers an unlimited amount of times until 30 minutes had passed and the training ended. To proceed to the next phase, the rat had to obtain an average of at least 50 rewards over two completed sessions. In case a rat obtained a lower number of rewards during the first two sessions, it was further trained on subsequent days until the criterion was met. In the next phase a trial started with the presentation of the left lever, the right lever, or both levers and pressing either lever was reinforced under a FR1 schedule. In this phase, levers retracted after a response was made, and an ITI of five seconds commenced while the house light remained illuminated. When all animals made at least 100 responses in a session during this phase, they progressed to the experiment.

Probabilistic Reversal Learning Task: The protocol used for this task was based on that of a previous study (Bijlsma et al., 2022). At the start of each session, one of the two levers was randomly selected to be 'correct' and the other 'incorrect'. A response on the 'correct' lever resulted in the delivery of a reward in 80% of trials, whereas a response on the 'incorrect' lever was reinforced in only 20% of trials. Each trial started with an ITI of five seconds, followed by illumination of the house light and the insertion of both levers into the chamber. After a 'correct' response, both levers retracted but the house light remained illuminated. An 'incorrect' response or a failure to respond within 30 seconds after lever insertion (i.e., omission), lead to retraction of both levers and extinction of the house light so that the chamber returned to its ITI state. When the rat made a string of eight consecutive trials on the 'correct' lever, the 'correct' lever was converted to the 'incorrect' lever and vice versa. This pattern repeated over the course of a daily session. Daily sessions were completed upon performing 200 trials or after 60 minutes had passed, whichever occurred first. The number of reversals made during a session was solely limited by the number of trials in a session.

Trial-by-trial analysis: This analysis was performed to assess the shifts in choice behaviour between subsequent trials, in order to investigate the sensitivity to positive and negative feedback. There are four different possibilities for behaviour per trial (i.e., win-stay, win-shift, lose-stay, lose--shift) for both the 'correct' and 'incorrect' lever. The number of times that each possibility occurred was summed per session and was subsequently converted to percentages.

Response Inhibition

Apparatus: Behavioural testing was executed in the aforementioned operant boxes.

Pre-training: Rats were first habituated for four days to the operant chamber for 30 minutes in which the house light was illuminated. 50 sucrose rewards were randomly delivered into the magazine with an average ITI of 15 seconds between reward deliveries. This phase was followed by a training phase in which, after an initial 20 seconds before the start of the first trial, rats had 40 seconds per trial to retrieve a sucrose pellet. The following trial commenced only after reward retrieval and the session ended after 60 trials.

Response inhibition: The protocol used for this task was modified from Verharen et al. (2019). In contrast to Verharen et al. (2019), where they adjusted the shock intensity to each individual, we used the same shock intensity for every rat. This experiment consisted of 60 trials of 40 seconds of which 30 were non-stimulus (NS) trials and 30 shock trials. In NS trials, rats were allowed to retrieve a reward directly after the drop of a sucrose reward. During the first 12 seconds in shock trials, a tone was produced by a speaker in the top right corner on the same wall as the house light. When rats collected the reward within this timeframe, they were punished with a footshock produced by a S/A aversive stimulator (Med Associates, USA). If rats did not retrieve the reward within 40 seconds in both NS and shock trials, the trial was labeled as an omission. RI was tested for six consecutive days followed by a rest day and another six consecutive test days. A shock intensity of 0,25mA was used during the first three days. Every three days the shock intensity was increased with 0,1mA until 0,55mA was used during the last three sessions.

Trial-by-trial analysis: Analysis of trials was performed to assess impulsivity. Both the amount of shocks and the shock index were used to measure impulsivity. The shock index was calculated by dividing the number of shocks by the amount of shock trials subtracted by the shock omissions (shocks/(shock trials- shock omissions)) Furthermore, successful reward retrieval, the amount of omissions and the latency to reward retrieval were used to evaluate overall performance in this task.

5-Choice-Serial-Reaction-Time-Task

Apparatus: Behavioural testing was conducted in operant conditioning chambers (Med Associates, USA, Product: MED-NP5L-D1) enclosed in sound-attenuating cubicles equipped with a ventilation fan. A food magazine, into which sugar pellets could be delivered via a dispenser, was located centrally on one side of the box. A white house light was mounted in the top-center on the same wall as the food magazine. Five apertures with a LED-light were located in a curved wall opposite of the house light. Online control of the apparatus and data collection was performed using MED-PC software (Med Associates, version 4.2.1.58).

Pre-training: The pre-training and the 5CSRTT protocol used were based on those of other studies (Achterberg et al., 2022; Amitai & Markou, 2011; Baarendse et al., 2013; Bari et al., 2008; Higgins & Breysse, 2008; Van Gaalen et

al., 2006). The first habituation phase existed of placing two sucrose pellets in both the food magazine and each of the five apertures. Then a session was started, which lasted 10 minutes until the rats ate all pellets provided. During the first five minutes, only the five aperture cue lights were on and in the last five minutes only the house light was illuminated. After two days a second habituation phase was initiated, which lasted three days. In this phase two sucrose pellets were placed in both the food magazine and all five apertures. After a habituation period of two minutes, in which the five cue lights were illuminated, a total of 80 pellets were dropped with a mean random interval of 15 seconds for 20 minutes to associate the sound of a pellet drop with receiving a reward. All lights were on during the pellet drop. The second habituation phase was followed by three training phases. All training phases started with a two minute habituation period, after which a 'free' pellet' dropped. Once the pellet was retrieved, the first trial began. After each trial, an ITI of five seconds occurred. In the first training phase all five aperture lights were illuminated. A nosepoke in one of the five apertures was rewarded with a sucrose pellet to link a nosepoke in an illuminated aperture with rewards. The training session ended after 100 trials or 27 minutes. When all rats retrieved 80 pellets within a session, they progressed to training phase two. In contrast to training phase one, now only one out of five apertures was illuminated in a pseudorandomized fashion. A correct response resulted into a reward delivery, while an incorrect response had no consequences. When all animals retrieved 80 pellets within a session, they moved on to training phase three. Here, a time-out (TO) of five seconds was introduced in case of an incorrect response, premature response or omission. A TO resulted in illumination of all lights and no reward, i.e. no pellet drop. After the TO, the lights were switched off indicating the ITI of five seconds. While the ITI and TO stayed consistent, the stimulus duration (SD) and limited hold (LH) were gradually reduced. The LH indicates the amount of time rats were given to retrieve a reward. When reward retrieval did not occur within the LH, the trial was labelled as an omission. When all rats achieved more than 80% accuracy and less than 20 omissions within a single session, they progressed to the following step. Table 1 shows the start and final settings and the gradual reduction of SD and LH over time. A session was ended either after 30 minutes or after a 100 trials. When all rats performed consistently over three consecutive sessions under the final settings, experimentation with daily challenges began.

5CSRTT and daily challenges: After the before mentioned three consistent days of testing under the final settings, the first daily challenge was introduced. Every daily challenge was followed by four baseline testing days. In the first daily challenge, the ITI was prolonged from five to ten seconds. Because of the longer ITI, the session ended after 45 minutes instead of 30 minutes. A shorter SD was used as the following challenge, where the SD was reduced from one second to half a second. The third daily challenge was the reduction of the ITI from five to two seconds. Finally, a variable ITI challenge was used, where the ITI varied pseudorandomly between two, four, six and eight seconds and with an average duration of five seconds. All other settings and session time remained equal to baseline testing conditions in these last three challenges.

	SD (sec)	LH (sec)
Start	16	18
2	8	10
3	4	6
4	2	4
5	1,5	3
Final	1	2

Table 1: During the last training phase of the 5CSRTT the SD and LH started on 16 and 18 seconds respectively. Over time both SD and LH would be reduced in steps until the final settings were reached. Only when rats achieved more than 80% accuracy and less than 20 omissions, they progressed to the next step.

Trial-by-trial analysis: Comparison between baseline testing and the following daily challenge were performed to assess cognitive flexibility, impulsive behaviour, sustained attention and processing speed. Impulsive behaviour was measured through accuracy and premature responses during the long ITI daily challenge. Sustained attention was assessed during both short ITI and short SD challenges through accuracy and omissions. With the variable ITI challenge, cognitive flexibility was assessed through accuracy, while processing speed was tested under all challenges through latency to reward and latency to correct response.

Elevated Plus Maze

The EPM was utilized during this experiment to investigate anxiety-like behaviour. The maze consisted of two open arms of 50 x 10 cm (L x W) and two closed arms of 50 x 10 x 40 cm (L x W x H). The arms were extensions of a central platform of 10 x 10 cm (L x W). The EPM was placed on 76 cm long legs to elevate the construction. This construction was located in a white light lit room, which resulted in a light intensity of 300 lux on the open arms and central platform, and 240 lux in the closed arms. A trial started with each rat being placed on the central platform with their nose pointed to the left open arm. Trials lasted five minutes for every rat

and the maze was cleaned between every trial with water and soap. All trials were divided over two days, to prevent interference of short social isolation. On the first day only the uneven numbered rats were tested and on the second day the even numbered rats. Cumulative duration in open arms, closed arms and central platform; entrees in both closed arms and open arms; and the mean velocity and total distance moved were assessed to evaluate anxiety-like behaviour. All data was acquired through video recordings (Logitech C920 camera) and were analyzed with tracking software (EthoVision, Version 9.0.718).

Large Open Field

Anxiety was also assessed through the lOF, where every rat was placed in a round arena with a diameter of 100 cm and 33,5 cm high walls. The open field was placed in a red-light lit room and one external white light source was placed on the ground, which resulted in a light intensity of 12 lux in the lOF. At the start of the trial, a rat was placed on a marked spot in the peripheral circle with its nose pointed towards the walls. A trial lasted ten minutes and the surface was cleaned with water and soap after each trial. To avoid interference of social isolation, testing was divided over two days in the same way as mentioned for the EPM. Cumulative duration in the peripheral, middle and central zone; entrees into all three zones; mean velocity; and total distances moved were measured for each individual. The peripheral, middle and central zones were created digitally in EthoVision (Version 9.0.718). Both the peripheral and central zones had a width of 20 cm, while the middle zone measured 10 cm and functioned more as a transition zone between the periphery and center. Data was obtained using tracking software as described for the EPM.

Small Open Field

The sOF was used to evaluate potential differences in locomotion between groups. Four boxes of 50 x 32,5 x 40 cm (L x W x H) were placed in a square. The room was lit with white light and the light intensity in the sOF boxes was 144 lux. Rats were tested in a pseudorandomized order and were placed in the middle of the box. The trial duration was 20 minutes and all boxes were cleaned with water and soap between trials. Mean velocity and total distance moved were measured. All data was acquired using tracking software as mentioned for the EPM test.

Statistical Analysis

All data was processed in Excel (version 2204), analysed in R studio (version 2022.02.2-485) and edited in Adobe Illustrator (version 26.2.1). Detailed statistical information of the figures are listed in the accompanying documents.

Probabilistic Reversal Learning: Behaviour in the PRL was analysed using a two-way repeated measures ANOVA (with sessions as within-subjects factor and group as between-subjects factor) followed up with Tukey tests for multiple comparisons. All graphs represent the mean ± standard error of the mean (SEM). An α of 0,05 was chosen as cut-off point for significance.

Response inhibition: As a repeated measures ANOVA cannot be used for count data, four different models were fitted to analyse the data: a (generalized) linear model and three different (generalized) linear mixed models (random intercept + random coefficient, random intercept, and random coefficient). Akaike Information criterions (AIC's) were calculated for each model to determine which model fitted the data best. Then, an ANOVA was used to obtain p-values of both main effects group and shock intensity and their interaction in this model. Animal and Session were used as random intercept and random coefficient respectively. For discrete dependent variables, such as the amount of shocks, omissions and successes, a Poisson distribution and the glm or glmmTMB function (package glmmTMB) were used. For the shock index, a continuous variable, assumptions for a gaussian distribution were tested by plotting the residuals. Since the residuals looked approximately normally distributed, a gaussian distribution and the lm and lmer function (package lme4) were used. When a main effect or interaction was significant, multiple comparisons were obtained through the summary() command. Additionally, confidence intervals were calculated with the confint() function. Survival analysis was performed for all latency data, because of the possibility to censor omitted trials. Since a proportional hazard model did not fit the data well, an accelerated failure model was used through de function survreg (package survival).

5CSRTT: Differences in baseline performance between groups were evaluated. Linear models with a gaussian distribution were made for continuous variables and generalized linear models with a Poisson distribution for discrete variables. Homogeneity of variance was tested with a Levene's test accompanied by a Shapiro-Wilk test to check for normality. When a parameter had a p-value of > 0,05 in both tests, a one-way ANOVA was used to test group differences in baseline performance. If these criteria were not met, a Kruskal-Wallis test was used for the same purpose. Comparisons between baseline and daily challenge performance were executed in the same manner as discussed for the RI task.

EPM, lOF and sOF: Homogeneity of variance, normal distribution of residuals, and comparisons between groups were executed in the same manner as mentioned for the baseline performance in the 5CSRTT. A Tukey HSD test was used as post-hoc test when necessary.

Results

Probabilistic Reversal Learning

In the PRL, rats performed better over time which was manifested in more correct responses, rewards, and reversals as well as fewer incorrect correct responses (Figure 3). Win-stay behaviour also increased over time, while lose-shift remained consistent over sessions. There was a significant effect of group on both the amount of rewards ($F_{3,400}$ = 4,32, p = 0,005) and win-stay behaviour ($F_{3,400}$ = 4,44, p = 0,004). Multiple comparisons revealed that both risky play groups earned more rewards than CTL rats (CTLR: p = 0,005; SPDR: p = 0,031). These differences can be explained by increased win-stay behaviour in CTLR and SPDR rats compared to the CTL group (p = 0,022 and p = 0,008 respectively). There were no additional group differences found during this task. While supplementation of risky play resulted in slightly better performance and higher sensitivity to positive feedback in socially housed rats, this effect was not evident in social play deprived rats. In contrast to CTLR and CTL rats, SPD and SPDR rats did not differ significantly in amount of rewards (p = 0,843) and win-stay behaviour (p = 0,864).

5-Choice-Serial-Reaction-Time-Task

To further investigate cognitive function, the 5CSRTT was executed. To asses consistent performance, a repeated measures ANOVA was performed, which showed that both accuracy ($F_{2,134}$ = 0,66, p = 0,518) and omissions ($F_{2,134}$ = 2,17, p = 0,118) were consistent over the three days prior to testing. Baseline performance did not differ between groups, which shows that all groups were able to learn the task with the protocol used in this study. Exposing the rats to the four daily challenges (long ITI, short SD, short ITI and variable ITI) affected their omissions, premature responses and percentage of correct responses significantly, resulting in deteriorated performance (Figure 4). Overall, omissions and premature responses increased, while the percentage of correct responses decreased compared to baseline conditions. The only exception is a decrease in premature responses when the ITI was shortened (p < 0,001). Although there was no significant overall group effect for these variables under all challenge conditions (Table 2), post-hoc tests showed differences in behaviour between days for CTL an SPD groups compared to CTLR and SPDR groups during the variable ITI challenge. CTL and SPD groups had significantly more omissions (CTL: z = 2,21, p = 0,027; SPD: z = 2,39, p = 0,017) and a significantly lower percentage of correct responses (CTL: z = -2,01, p = 0,044; SPD: z = -2,83, p = 0,005) compared to their baseline performance. Unlike both low risk groups, both CTLR and SPDR groups showed no significant difference in performance between baseline and variable ITI conditions on these variables. The overall performance of the risky play groups were however not better than those of the CTL and SPD groups during this challenge. In addition to these findings, there were no evident patterns found, regarding the effect of daily challenges on latency to reward and latency to correct response. These results indicate no differences between groups in impulsive behaviour, sustained attention and processing speed. Risky play groups however seemed less affected in their performance compared to CTL and SPD groups during the variable ITI challenge, which could indicate altered cognitive flexibility. Nevertheless, these behavioural differences did not result in overall better performance during this daily challenge.

	Daily challenges	LR χ^2	P - value
Omissions	Long ITI	6,48	0,091
	Short SD	0,75	0,860
	Short ITI	0,39	0,942
	Variable ITI	7,22	0,065
Premature Responses	Long ITI	2,26	0,521
	Short SD	2,79	0,426
	Short ITI	1,95	0,583
	Variable ITI	2,74	0,434
Percentage of Correct Responses	Long ITI	2,06	0,561
	Short SD	0,31	0,958
	Short ITI	0,53	0,913
	Variable ITI	5,18	0,159

Table 2: Results of the analysis of main effect “Group” for omissions, premature responses and percentage of correct responses. There were no significant group differences found for all variables and all daily challenges.

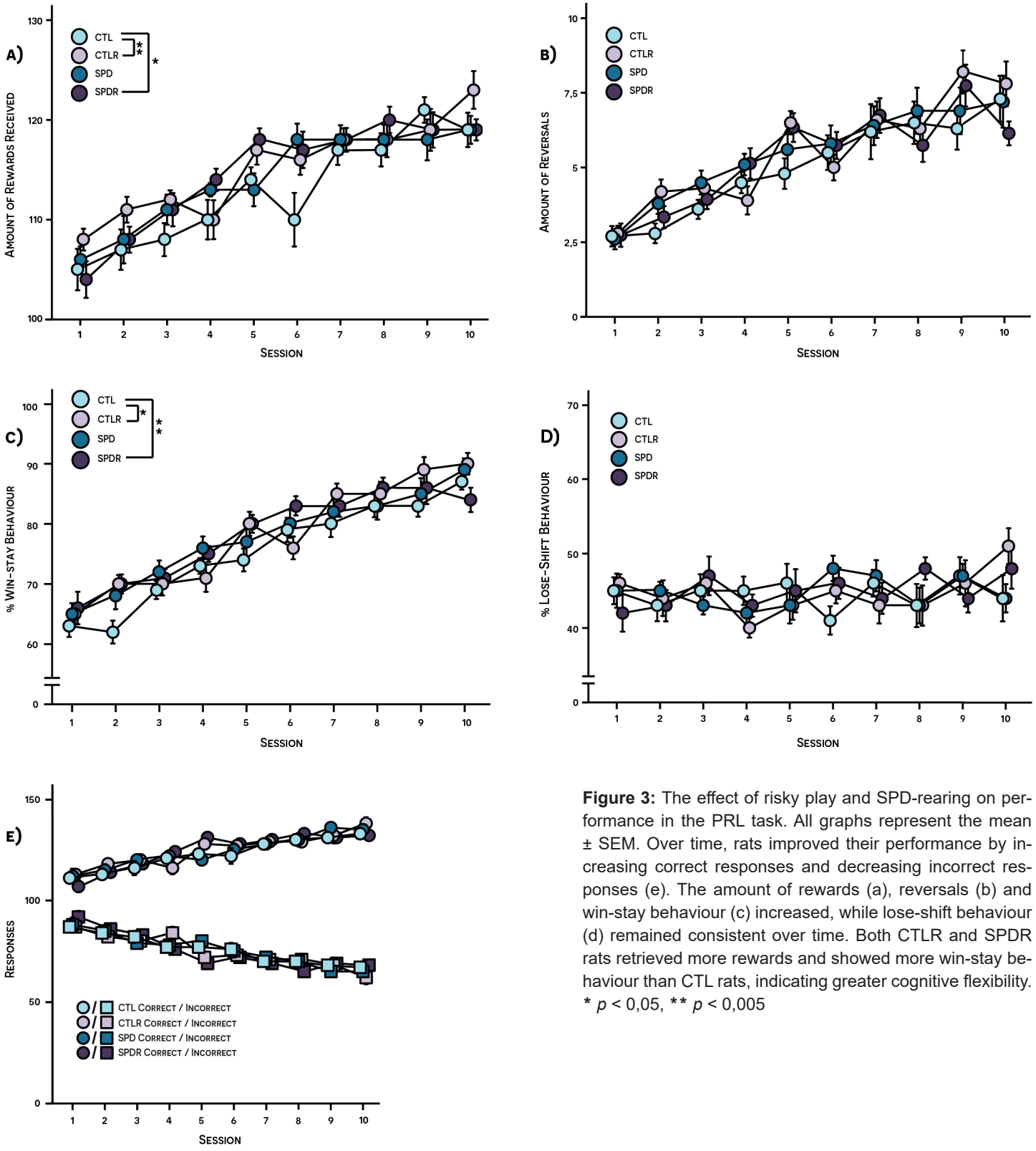


Figure 3: The effect of risky play and SPD-rearing on performance in the PRL task. All graphs represent the mean ± SEM. Over time, rats improved their performance by increasing correct responses and decreasing incorrect responses (e). The amount of rewards (a), reversals (b) and win-stay behaviour (c) increased, while lose-shift behaviour (d) remained consistent over time. Both CTLR and SPDR rats retrieved more rewards and showed more win-stay behaviour than CTL rats, indicating greater cognitive flexibility. * $p < 0,05$, ** $p < 0,005$

Response Inhibition

Impulsive behaviour was also evaluated with the RI task. Data from this task with a shock intensity of 0,25mA showed a high amount of shocks and omissions as well as a low amount of successes (data not shown). This could indicate that this amperage was insufficient to establish a clear distinction between shock and non-stimulus trials, resulting in bad performance. For this reason, data under this condition was left out of the analysis. Assessment of the remaining data with shock intensities 0,35mA, 0,45mA and 0,55mA showed a significant effect of shock intensity on successes, omissions and shocks (Figure 5). Raising the mA, with a limit of 0,55mA, resulted in better performance through more successes ($p < 0,001$) and fewer shocks ($p < 0,001$) reflecting increased inhibition. This increase in total successes was a result of better performance during shock trials ($p < 0,001$), which stagnated between 0,45 and 0,55mA. Furthermore, there was a trend for rearing conditions on the amount of shocks ($p = 0,1$). Although multiple comparisons are not appriopriate in case of a trend, de-

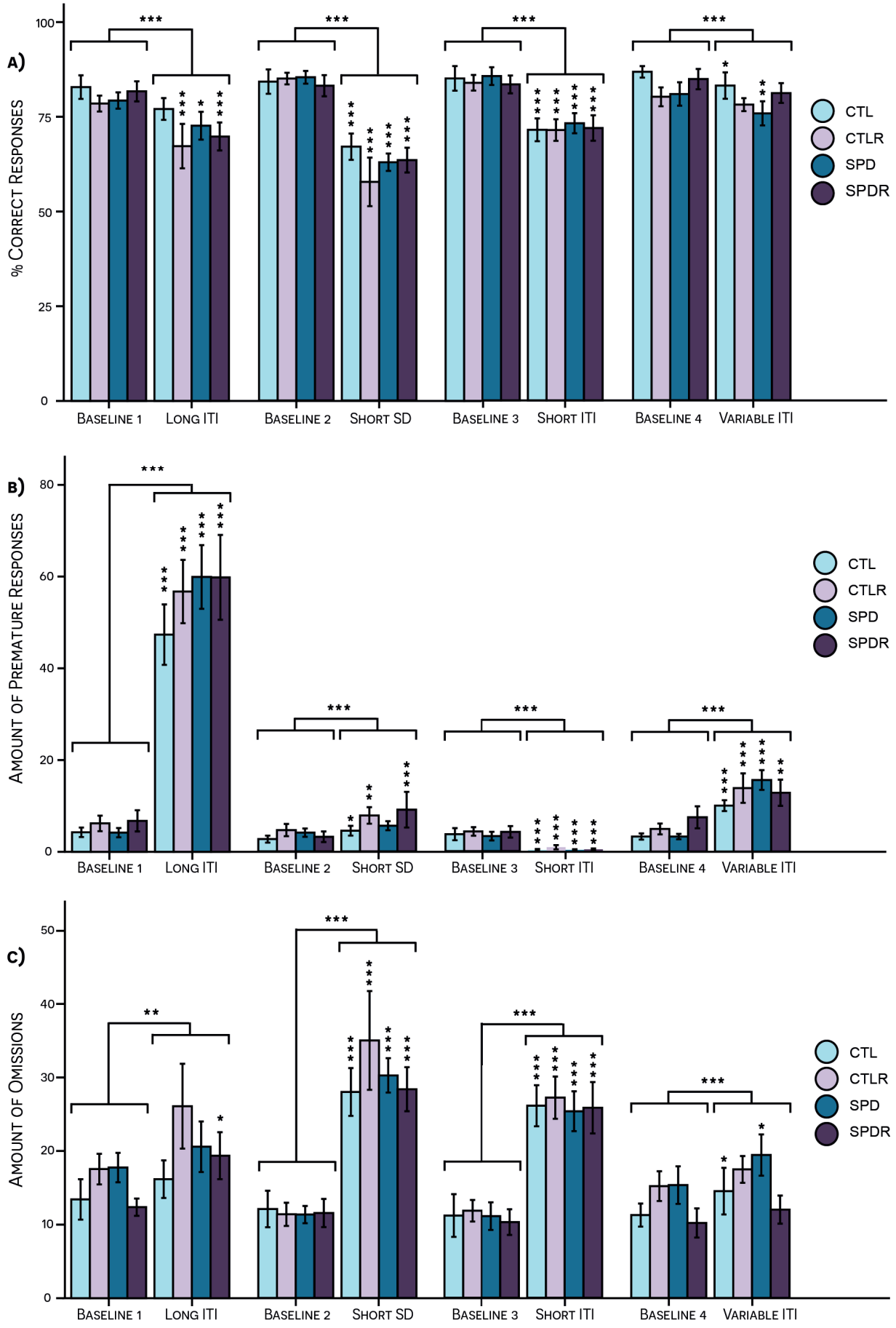
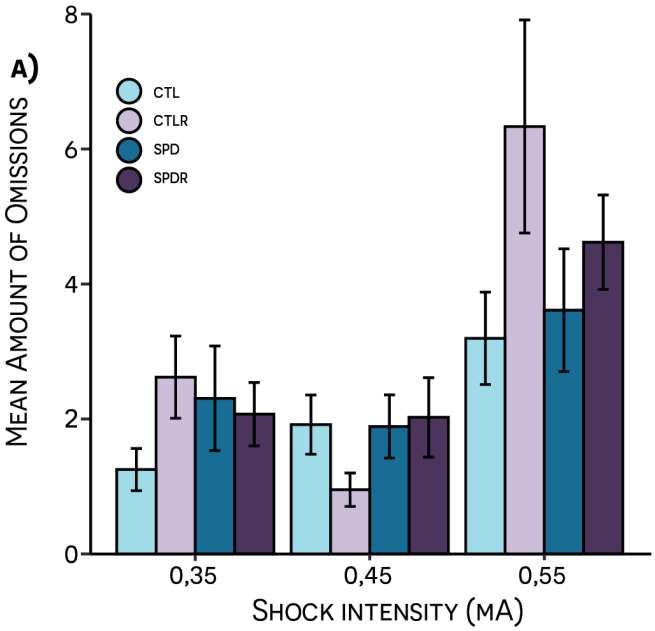
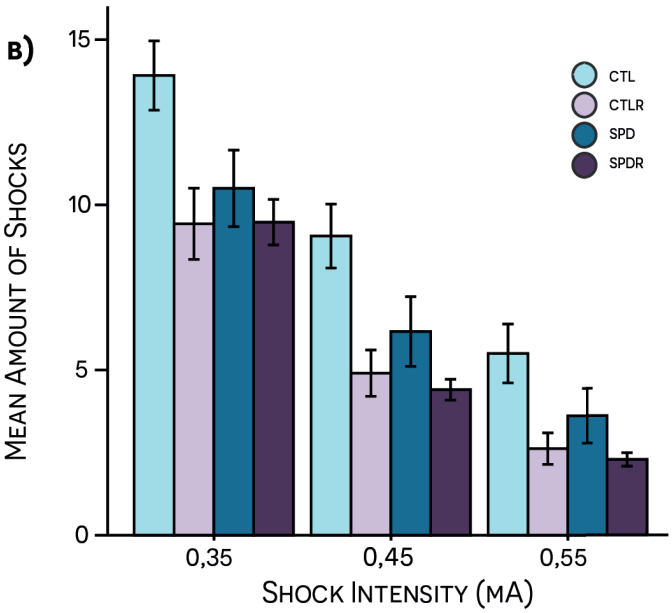


Figure 4: The effect of risky play and SPD-rearing on performance in the 5CSRTT. Each graph represents the mean ± SEM. All challenges resulted in less correct responses (a) and more omissions (c) compared to their baseline performance. The variable ITI challenge did not affect the percentage of correct responses and amount of omissions in CTLR and SPDR rats, while the performance of CTL and SPD were significantly affected. Subjecting the rats to the different challenges also resulted in significantly more premature responses (b), except for the short ITI challenge, where premature responses were significantly lower. Large brackets with asterisks indicate significant overall differences between days. Vertically stacked asterisks above individual bars show the results of multiple comparisons for main effect "day." Here an asterisk indicates a significant difference between baseline and challenge performance per group. * $p < 0,05$, ** $p < 0,005$, *** $p < 0,001$





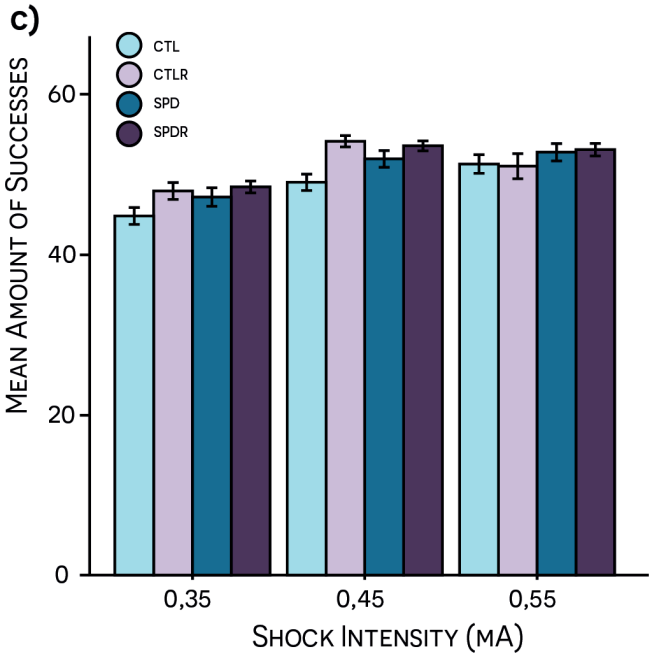
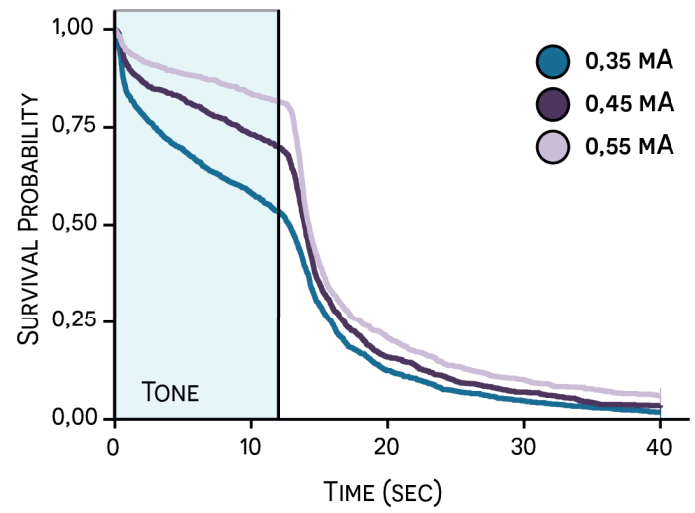


Figure 5: The effect of risky play and SPD-rearing on performance in the RI task. All graphs show the mean ± SEM. The overall effect of shock intensity was significant for the amount of omissions (a), shocks (b) and successes (c). The amount of omissions increased significantly from 0,35mA to 0,55mA. Amplifying the shock intensity from 0,35mA to 0,55mA decreased the amount of shocks. The amount of successes increased significantly from 0,35mA to 0,45mA, but did not increase further from 0,45mA to 0,55mA. There were no significant differences in performance between groups. Since the multiple comparisons between shock intensities were performed per group, no overall significance symbols could be displayed in these graphs. Detailed statistical information can be found in the accompanying documents.



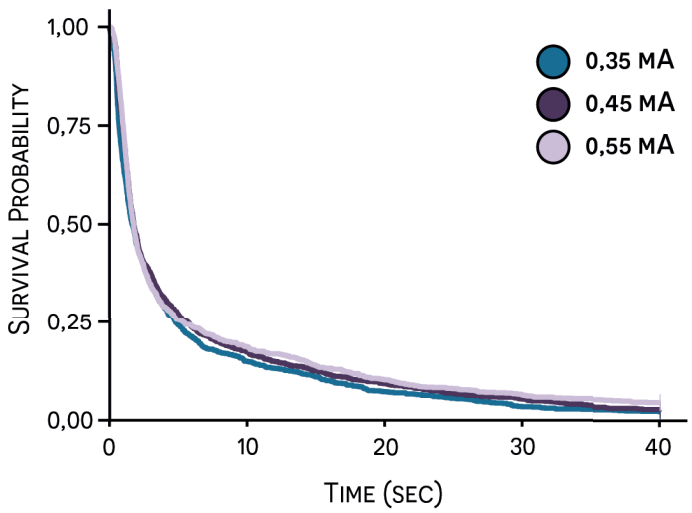


Figure 6: The effect of risky play and SPD-rearing on latency to reward during shock trials (a) and NS trials (b). Since there were no significant overall groups differences, the latency of only CTL rats are displayed in this graph to illustrate the effect of shock intensity. While shock intensity did not alter the latency to reward during NS trials, latency to reward became significantly longer with increasing shock intensities during shock trials, resulting in lower amounts of footshocks.

scriptive data shows that CTL rats received more shocks than all other groups under all shock intensities. In addition to better performance, amplified shock intensities resulted in increased omissions ($p < 0{,}001$) and longer latencies to reward during shock trials (Figure 6). This indicates that higher amperages result in more aversion to this stimulus.

Taken together, these findings show that increasing the shock intensity to a limit of 0,55mA improves performance in all groups. Since only a trend was found for rearing conditions on the amount of shocks, our findings suggest that both risky play and social play deprivation do not have a clear effect on impulsive behaviour in this set-up.

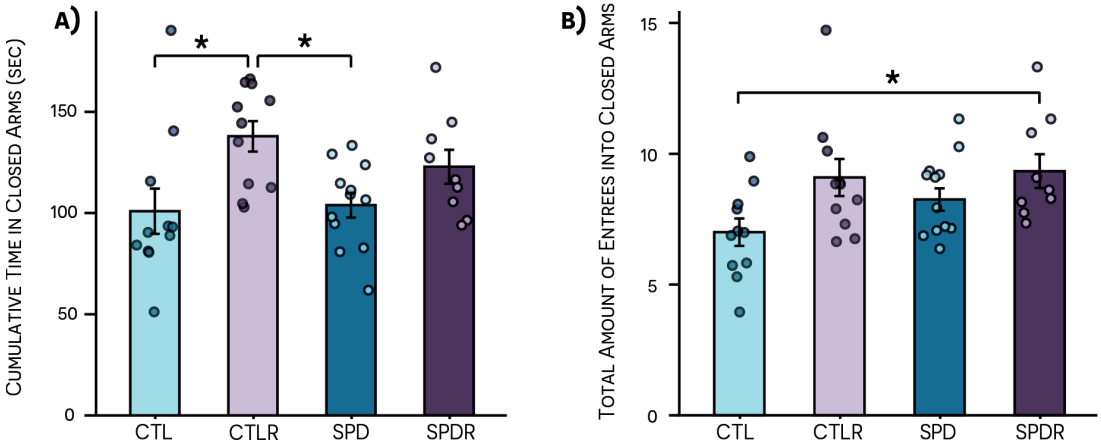


Figure 7:
Behavioural outcomes in the EPM. These graphs represent the mean ± SEM. Individual data points are displayed as coloured circles. CTLR rats stayed longer in the closed arms than CTL and SPD rats (a). Additionally, SPDR rats made more entrees into the closed arms compared to CTL rats (b).
* $p < 0{,}05$

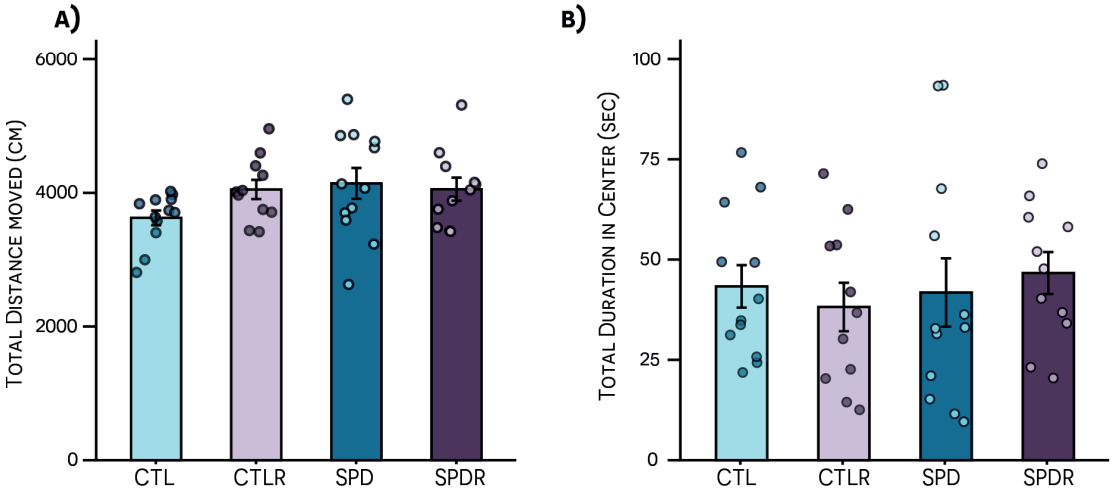


Figure 8:
Behavioural outcomes in the large open field. These graphs represent the mean ± SEM. Individual data points are displayed as coloured circles. In contrast to the small open field, no differences between groups were found in total distance moved (a). There was also no distinction between groups regarding their time in the central zone (b).

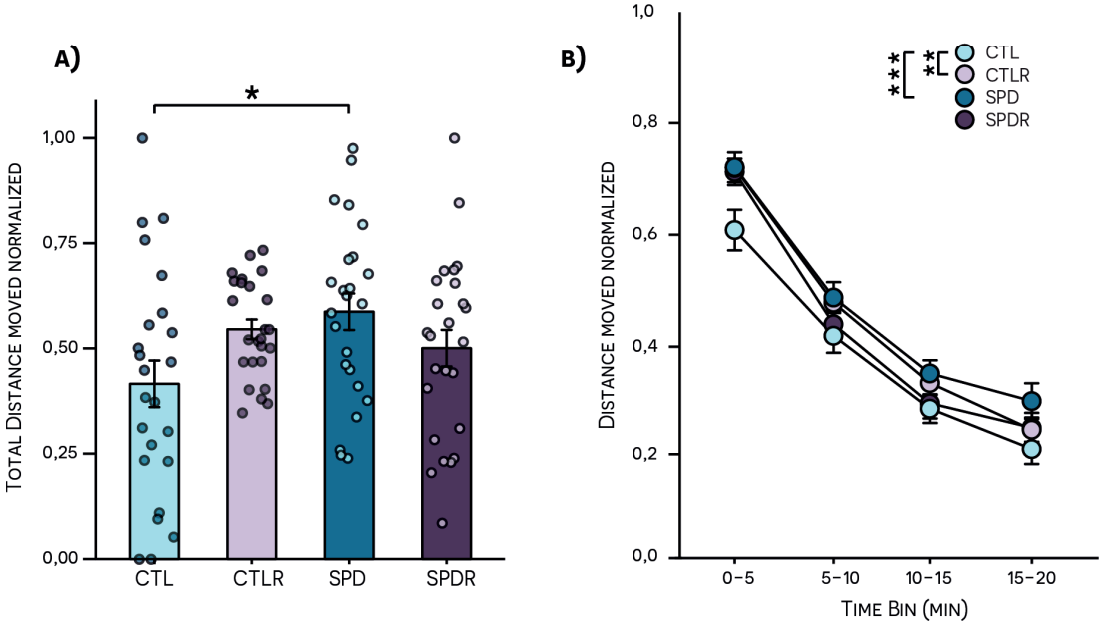


Figure 9:
Locomotion differences in the small open field. These graphs represent the mean ± SEM. Individual data points are displayed as coloured circles. SPD rats covered significantly more distance than CTL rats regarding the whole test (a). When divided in time bins, both CTLR and SPD rats covered more distance than CTL rats (b).
* $p < 0{,}005$, ** $p < 0{,}005$, *** $p < 0{,}001$

Elevated Plus Maze and Large Open Field

To assess anxiety-like behaviour, an EPM and lOF were used. During EPM experimentation, one rat from the CTL and two from the SPDR group jumped of the construction. These rats were excluded from the analyses, since two of those rats had extreme values for most variables. The EPM showed significant differences between groups for the amount of entrees ($F_{3,39}$ = 3,26, p = 0,032) and the total duration ($F_{3,39}$ = 4,32, p = 0,010) in the closed arms. A Tukey test revealed that the CTLR group stayed longer in the closed arms compared to CTL (p = 0,017) and SPD (p = 0,027) groups (Figure 7). Furthermore, multiple comparisons showed significant more entrees into the closed arms for the SPDR group compared to the CTL group (p = 0,043). The longer duration and increased amount of entrees in the closed arms could not be explained by differences in locomotion since there was no significant distinction between groups for their mean velocity ($\chi 2$ = 1,10, p = 0,776) and total distance moved ($\chi 2$ = 1,10, p = 0,776). In contrast to the closed arms, there were no group differences found for duration and entrees into the open arms ($F_{3,39}$ = 1,38, p = 0,262 and $F_{3,39}$ = 1,16, p = 0,338 respectively). The preference of risky play rats for the closed arms could indicate increased anxiety-like behaviour. However, this did not result in less activity on the open arms, which contradicts the former explanation. Like the EPM, no significant differences were found for the mean velocity ($F_{3,42}$ = 2,01, p = 0,127) and total distance moved ($F_{3,42}$ = 1,90, p = 0,144) in the lOF (Figure 8). Frequencies and entrees into the different zones did not differ significantly between groups, which would indicate that all groups have similar anxiety levels under these conditions.

Small Open Field

Finally, a smaller open field was used to evaluate group differences in locomotion. Both batch two and three performed this task. Since batch two covered more distance than batch three, assumably because they were younger at testing, data was normalized. This data showed that the SPD group covered significantly more distance than the CTL group (p = 0,030, see Figure 9). When data was analyzed in bins of five minutes, group differences were found as well ($F_{3,376}$ = 22,58, p < 0,001). Here, SPD and CTLR groups covered more distance compared to the CTL group (p < 0,001 and p = 0,004 respectively). Although these findings suggest hyperlocomotion in SPD and CTLR groups, this was not reflected in their performances during the previously described tasks.

Discussion

This study is one of the first to examine the effect of both social play deprivation and risky play during p21-42 on cognitive performance and anxiety in rats. The PRL task, 5CSRTT and RI task were used to evaluate multiple facets of cognitive function. The PRL task demonstrated a greater cognitive flexibility for CTLR rats compared to CTL rats, reflected by more rewards and increased win-stay behaviour for CTLR rats. Similarly, both risky play groups were less affected by the changed settings in the variable ITI challenge in the 5CSRTT, indicating greater cognitive flexibility as well. Anxiety-like behaviour was assessed through a lOF and EPM. While the large open field did not produce a distinction between groups, CTLR and SPDR displayed altered behaviour in the EPM indicating a preference for the closed arms. Finally, a smaller open field was used to evaluate potential locomotion differences. In this test, SPD rats exhibited hyperactivity compared to CTL rats.

Consistent with previous studies, rats from all groups were able to learn the PRL task with increased correct responses, rewards and reversals as well as decreased incorrect responses over sessions (Amitai et al., 2014; Bijlsma et al., 2022). While Bijlsma et al. (2022) observed that SPD rats made more reversals as well as win-stay choices, and were less able to change tactics after a reversal than CTL rats, the current data revealed no distinction between SPD rats and the other groups. Lack of significant differences between CTL and SPD animals in our study, can be a result of smaller group sizes. Nevertheless, our study showed that supplementation of risky play resulted in increased rewards and win-stay behaviour in pair-housed rats, indicating better performance and cognitive flexibility for risky play animals. This effect was however not evident for SPD-reared rats, which might be due to small group sizes as well. It could also suggest that risky play supplementation is less effective in improving performance and cognitive flexibility in social play deprived rats. A possible explanation could be that experiencing risky play opportunities without a peer is less effective than playing together in the risky play cage.

In addition to our findings in the PRL task, we found that risky play had an effect on cognitive flexibility in the 5CSRTT. In accordance with other studies, both SPD and CTL rats showed decreased performance and increased impulsive behaviour during the long ITI, variable ITI and short SD challenges, while impulse control was better in the short ITI challenge (Dalley et al., 2002; Liu et al., 2017). In addition to these findings, risky play did not affect overall performance or impulsive behaviour under baseline conditions. However, both CTLR and SPDR groups were less affected by the variable ITI challenge than CTL and SPD groups. There was no distinction between days in omissions and percentage of correct responses for the risky play groups, while low risk groups had more omissions and a lower percentage of correct responses during the challenge. This could indicate that providing risky play opportunities during

the juvenile phase results in greater cognitive flexibility in adolescence.

Akin to the 5CSRTT, all groups had similar performance reflected in a comparable amount of successes and omissions. There was however a trend for rearing conditions on the amount of shocks. Absence of significance is most likely due to the conservative mixed models used in this study. Descriptive data shows that CTL rats tended to receive more shocks than all other groups. Besides suggesting differences in impulsivity, this could also indicate a different way of responding to footshocks. Previous work on the effect of isolation on responses to footshocks were however ambiguous (Arakawa, 2002; Lukkes et al., 2009; Nishikawa & Tanaka, 1978). Two of these studies found that isolated rats showed more freezing and jumping behaviour as a reaction to a footshock, while the other observed more jumping behaviour in socially housed rats. Since impulsive control in CTL rats did not differ from the other groups during the 5CSRTT, it is less probable that altered impulse control is the cause of this trend.

To assess differences in anxiety-like behaviour, a IOF and EPM were used. In accordance with Weiss et al. (2004), our data showed no group differences in mean velocity, total distance moved, and activity in the central zone in the large open field under dim light conditions. Despite no altered locomotion in the large open field, the smaller open field revealed hyperactivity for SPD rats, which has been observed in other studies as well (Dalley et al., 2002; Del Arco et al., 2004; Hellemans et al., 2004; Liu et al., 2017). In contrast to our findings in the large open field, risky play animals behaved differently compared to the CTL and SPD groups in the EPM. Although open arm time and entrees did not differ between groups, CTLR rats stayed significantly longer in the closed arms compared to both low risk groups. Additionally, SPDR rats had more entrees into the closed arms in comparison with the CTL group. These findings suggest that risky play animals have a preference for the closed arms without reducing their activity in the open arms. It is not clear if this preference is a result of heightened anxiety levels or if other mechanisms cause this behaviour. Surprisingly, no differences between isolated and socially housed rats were found, which is not in agreement with studies where isolated rats show a decreased amount of time and entrees in the open arms (Hellemans et al., 2004; Kokare et al., 2010; Liu et al., 2017; Weiss et al., 2004; Yorgason et al., 2013).

Lack of differences between socially housed and isolated rats in this study could be a result of the social play deprivation method used. Here, social play deprived rats were placed in the same home cage with a perforated transparent divider, while other studies placed these rats in their own separate boxes. Since our set-up facilitates more close-up contact, our rearing method might ameliorate the effects of social isolation. Additionally, extensive handling of the animals during cognitive behavioural experimentation could mitigate anxiety-like behaviour. Pritchard et al. (2013) found, indeed, that repeated handling eliminated the difference between isolated and socially housed rats in open arm time in the EPM, mainly through decreased open arm time in socially housed rats. Furthermore, absence of group differences in the larger open field could be a consequence of the low light intensity in our set-up. Besides having no effect on anxiety, social play deprivation had no impact on performance in the different cognitive tasks. This could be explained by the interplay between increased food reward motivated behaviour and impaired cognition in isolated rats (Fone & Porkess, 2008). Despite no evident effect of social play deprivation on cognition and anxiety, supplementation of risky play opportunities seemed to slightly increase cognitive flexibility for socially housed rats. However, these effects were not found for other cognitive functions or in the social play deprivation group. A conceivable explanation is that either the risky play cage did not provide enough risk to induce behavioural changes or that play times and frequency were not sufficient. Therefore, it might be beneficial to house risky play rats into the risky play cages themselves, instead of providing only 30 minutes twice a day. This way rats are free to choose when and how often they want to experience risky play.

Other considerations to improve our experimental set-up are to only use 0,45mA in the RI task and to adjust the criteria to start experimentation in the 5CSRTT. Using multiple shock intensities made group comparisons of overall performance more complex than necessary. Since rats performed best under 0,45mA conditions, it is recommended to use this amperage and increase the amount of sessions to at least five. Then, the criteria of >80% accuracy and <20 omissions proved to impede progression to experimentation in the 5CSRTT, since not all rats were able to reach these criteria. Instead, using consistent performance as a benchmark would be more useful.

Taken together our study shows that risky play supplementation during p21-42 results in better cognitive flexibility for socially housed rats. Additionally, providing risky play opportunities seem to increase anxiety-like behaviour in the EPM. These findings indicate that risky play could lead to enhanced cognitive development. However, some expected behavioural changes were not observed, which could be a result of our rearing method and the means of risky play supplementation. Further research is needed to evaluate the influence of providing more risky play opportunities on differences in cognitive performance and anxiety. Moreover, comparisons of often used isolation-rearing methods and our social play deprivation method would pro-

vide new insights on their influence on fear and cognitive development

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