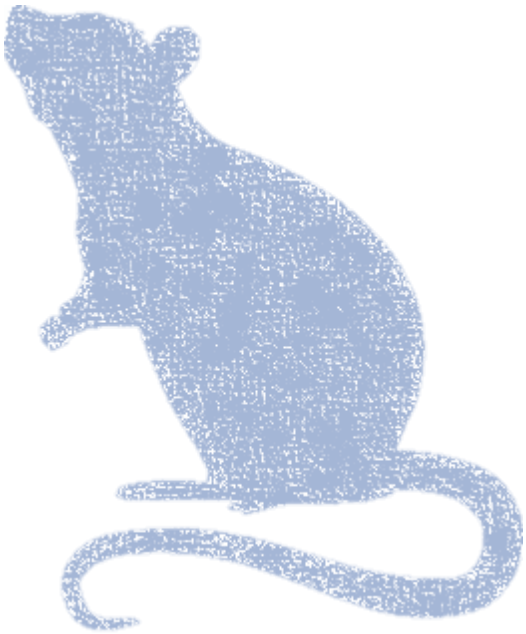
Utrecht University

Research Project

Do social play deprived rats have a different responsivity to stress?

The importance of social play behaviour for stress resilience



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Abstract

Background
Social play behaviour is a healthy form of interaction during the early phases of life of humans and most non-human mammals. Social play is thought to be important for the regulation of stress responses and appropriate behavioural responses to changing social situations in adulthood. In this study, the effects of social play deprivation on behaviour and stress responsivity were investigated. Two stress tests were performed, in which the corticosterone response to stressful stimuli has been determined in social play deprived (SPD) rats and was compared to control rats that were not isolated during early development.

Conclusion
There was no significant difference in the responsivity to stress between SPD rats and control rats. However, the increase of the plasma corticosterone level between baseline and at 15 minutes after exposure to social stress was found to be moderately positively correlated with a few behavioural acts for the SPD group, including the total freezing time and the total amount of submissive posture, but not for the control group. A positive correlation was also found between the amount of clinch attacks and the total freezing time of the SPD group, though this correlation was not significant for the control group. This may indicate that social play deprivation causes a higher sensitivity of the hypothalamic-pituitary-adrenocortical (HPA) axis.

Furthermore, there were significant differences among the various time points of the corticosterone response in both stress tests. There was also a statistically significant interaction between the effects of the experiment day on plasma corticosterone levels after exposure to a novel environment. This demonstrates the dynamics of the corticosterone response and suggests a sensitivity of corticosterone to stressors and environmental factors.

Keywords

Social play, play deprivation, behaviour, stress, corticosterone

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Introduction

Aim of the study

Social play is of vital importance for healthy development of humans and most non-human mammals, as it allows to practice physical and communicative skills (Nijhof et al., 2018). Most of the knowledge about the functional relevance of play is derived from research in rodent models. Rats display social play behaviour that can easily be observed and measured in a laboratory setting. Rats that were socially isolated during the stage of their lives in which they play most (play deprivation) displayed enhanced anxiety-like behaviour and impairments in cognitive flexibility (Trezza et al., 2010).

Furthermore, early-life play behaviour is important for the regulation of the stress response and appropriate behavioural response to changing social situations in adulthood (Von Frijtag et al., 2002). Evidence indicated that social isolation alters the regulation of the hypothalamic-pituitary-adrenocortical (HPA) axis component of the stress response system, involving the effective glucocorticoid feedback inhibition being affected. In some studies, social isolation (21 days to 18 weeks) is associated with significantly larger corticosterone responses to acute stress in mice, rats and hamsters due to poor regulation of stress reactivity and increased stress reactivity. However, other studies associated long-term social isolation with decreased corticosterone responses (Hawkley et al., 2012).

Although it has been theorized that play behaviour contributes to the development of brain and behaviour, there is still little factual evidence to support this. For example, it remains unclear how social play affects the development of resilience, the ability to adapt and social skills. Alterations to stress response systems resulting from the early rearing environment may have specific effects on the development of brain structure and capacity that affect the self-regulation of behaviour (Blair, 2010). Nonetheless, the factors that modulate play behaviour remain poorly understood.

The aim of this study is therefore to determine the relation between social play behaviour and stress responsivity. To that end, a total of two stress tests were performed with two groups of rats, involving a group of rats that were deprived from social play (SPD) at two weeks of age for three weeks and a group of control rats that were not isolated during early development. Consequently, the behavioural data and corticosterone response to stressful stimuli of these groups were determined and compared against each other (Figure 1). The stress responsivity will give an indication about the general resilience to stress.

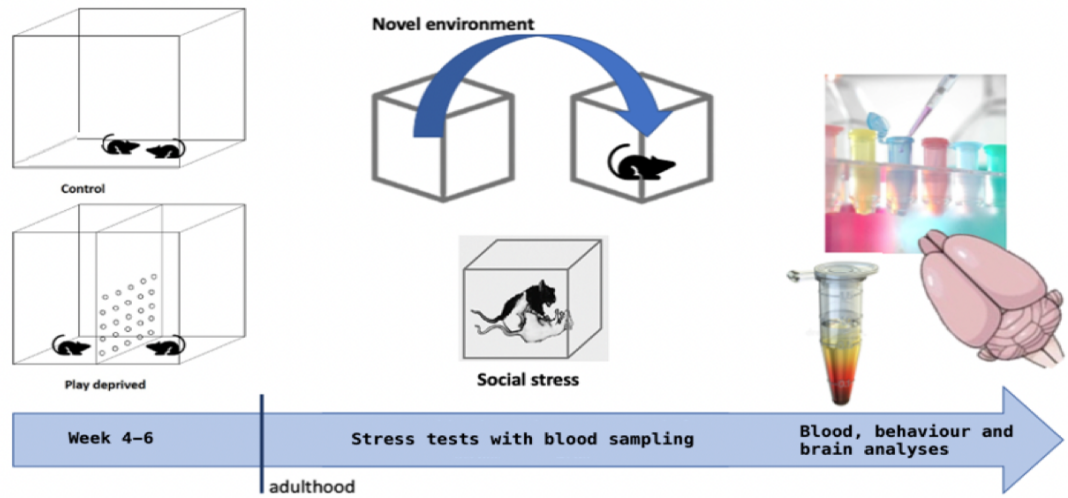

Figure 1. Schematic overview of the research project. Two stress tests were performed, involving the control and SPD rats being exposed to (1) a novel environment and (2) social stress.

Hypothesis

H_0 = There is no significant difference in the responsivity to stress between social play deprived rats and control rats.

H_a = There is a significant difference in the responsivity to stress between social play deprived rats and control rats.

Materials and Methods

Subjects

Male Lister Hooded rats (n = 32) were used in both experiments. The rats arrived in the facility lab (GDL, Utrecht, The Netherlands) late august at two weeks of age, with their dams. They were housed in 40 x 26 x 20 cm (l x w x h) Macrolon cages with primary enrichment, including wood shavings, shelter and a wooden block (Figure 2). Food and water were available *ad libitum*. Animals were housed in temperature-controlled rooms (21 ± 2°C) under a normal light:dark cycle (lights off at 9pm). The rats were acclimatized for a week, after which they have been weaned. All experimental procedures were carried out between 9:00 a.m. and 3:00 p.m. and were approved by the Animal Ethics Committee of Utrecht University.

Figure 2. Cages used for social housing.

Social play deprivation paradigm

After acclimatization, the rats were assigned to one of two groups, i.e., a group of social play deprived (SPD) rats (n = 16) and a group of control rats (n = 16). The SPD rats were placed in sibling pairs in cages with a synthetic, perforated partition, which allowed the rats to see, smell and hear each other, but prevented the rats from having physical interaction, thus effectively depriving them from social play behaviour. The control rats were sibling pair housed without partition, but otherwise handled similarly as the SPD group. After three weeks all SPD rats were socially housed again by removing the partition, the control rats stayed housed in the same pairs until adulthood. From then, the rats were handled and habituated briefly for 3-4 minutes, followed by the mimic of the tail incision using a paperclip two times per week. This was done to minimize the effect of the tail incision on the stress response. Routinely handling would ease this problem and therefore, more reliable baseline stress levels can be maintained.

First stress test: novel environment
At 77 days old, the novel environment stress test was performed. In this test the rats are solitary exposed to a clean, empty cage with water and food, for three hours. This stress test was spread over four days. Blood samples were collected before the stress onset, and then at 15, 30, 45, 60, 90, 120 and 180 minutes after exposure to the novel environment.

Second stress test: social stress
Two weeks after the first stress test, the rats were exposed to social stress. The resident intruder protocol was used for exposure to social stress. The entire interaction was recorded on cameras (Logitec C922 Pro Stream webcam).

Individually housed male Wistar rats (n = 7) were used as residents. These rats were originally used as breeders and were characterized for their offensive behaviour. A Lister Hooded rat (intruder) was introduced into one of the resident's home cage. After ten minutes, we removed the intruder from the cage.

This stress test was spread over eight days. Each day, two residents in their home cage were put in two different rooms. Four intruders were one by one introduced one-time to a resident. The interval between two social interaction periods was ten minutes. The intruders were isolated two hours until the exposure to social stress. Blood samples were again collected at baseline, 50 minutes before the social interaction, and at 15, 30, 45, 60, 90, 120 and 180 minutes after the end of the social interaction. The experimental rats were housed temporarily in their isolation cage during the sample collections to avoid social interaction with other rats.

The recordings of the interactions were used to score the latency, frequency and duration of behaviour of both the intruder and the resident, using the programme 'Observer XT 15.0'.

For the intruder, a total of 7 behavioural acts and postures were scored and grouped into the following behavioural categories: (1) *Moving towards*; (2) *Social exploration* (i.e., crawl over, nosing, investigating opponent, anogenital sniffing, social groom); (3) *Non-social exploration* (i.e., rearing, scanning, digging); (4) *Freezing*; (5) *Upright posture*; (6) *Clinch attack (initiated by the resident)*; (7) *Submission*.

For the resident, a total of 9 behavioural acts and postures were scored and grouped in the following behavioural categories: (1) *Moving towards;* (2) *Ano-genital sniffing*; (3) *Social exploration* (i.e., crawl over, nosing, investigating opponent, social groom); (4) *Non-social exploration* (i.e., rearing, scanning, digging); (5) *Inactivity*; (6) *Upright posture; (7) Lateral threat;* (8) *Clinch attack*; (9) *Keep down*.

Intruder	Resident
Moving towards	Moving towards
Social exploration	Ano-genital sniffing
Non-social exploration	Social exploration
Freezing	Non-social exploration
Upright posture	Inactivity
Clinch attack	Upright posture
Submission	Lateral threat
	Clinch attack
	Keep down

Table 1. Overview of all behavioural acts and postures of the intruder and resident.

The behavioural data of this test were used to classify and compare the behaviour of the SPD and control rats.

After completion of the stress tests, the rats were sacrificed, and brains were collected and stored frozen for further analysis

Analysis of experimental data

Blood was sampled through the tail incision method and each sample contained a maximum of 150 uL blood. The blood samples were centrifuged on the same day and the plasma obtained was stored frozen (-40 °C), until they were processed for corticosterone assessment. ImmuChem™Double Antibody Corticosterone 125I radioimmunoassay (RIA) kit for rats and mice (MP Biomedicals LLC, Orangeburg, NY, USA) were used to measure plasma corticosterone in duplicate. RIAs are based on competing interactions between antibody and radiolabelled corticosterone. This method is cleared to be both sensitive and specific (Bekhbat et al., 2018).

Plasma was diluted 1:200 in assay buffer according to manufacturer protocol. For every stress test, a total of 512 samples were divided into six centrifuge runs. The Thermo Scientific™ Megafuge 40R centrifuge was used with a speed of 2500 RPM for 15 minutes. The tubes were read on a PerkinElmer's automatic gamma counter (2470 WIZARD2). The coefficient of variance among the duplicates equalled less than 10.0%. All statistical analyses were performed using GraphPad Prism 9 for macOS, with the alpha value set to 0.05. Corticosterone data were analysed using a two-way repeated measures ANOVA, whereby 'time' (before and at different time points after stress) counted as within-subject factor and 'group' (SPD vs. control) as between-subject factor. The specific day of the experiment, dams cage and new cage were included as covariates. Correlation between variables was calculated using two-tailed Pearson correlation using IBM® SPSS® Statistics (Version 27). The behavioural data of the social stress test were statistically analysed with the use of t-tests.

Results

The first and second stress tests resulted in corticosterone responses, and the SPD rats were compared to the control animals (Table 2). The data was found to be normally distributed according to quantile-quantile (Q-Q) plots. In the second stress test, one blood sample at 90 minutes after exposure was missing in a rat from the control group due to an insufficient blood sample.

	group	Mean	Std. Deviation	N
NE_A	0	131.602407	64.8205780	16
	1	93.5043803	45.1104165	16
	Total	112.553394	58.2435189	32
NE_B	0	212.345798	62.5811605	16
	1	221.284030	76.8927880	16
	Total	216.814914	69.1125014	32
NE_C	0	234.446173	71.9517783	16
	1	279.593251	131.096568	16
	Total	257.019712	106.522237	32
NE_D	0	228.199380	86.2319423	16
	1	270.007598	128.382171	16
	Total	249.103489	109.655231	32
NE_E	0	226.688320	156.305709	16
	1	240.619434	113.631264	16
	Total	233.653877	134.608835	32
NE_F	0	244.957967	201.658822	16
	1	186.512641	102.657305	16
	Total	215.735304	160.181237	32
NE_G	0	223.022937	169.082466	16
	1	154.016479	79.2488321	16
	Total	188.519708	134.540316	32
NE_H	0	50.2333330	28.8601072	16
	1	50.3179875	28.1760792	16
	Total	50.2756603	28.0564039	32

	group	Mean	Std. Deviation	N
SS_A	0	87.7018021	36.3104781	15
	1	107.976437	66.2928728	16
	Total	98.1661296	54.0252696	31
SS_B	0	146.289861	72.4504699	15
	1	164.203997	68.5876923	16
	Total	155.535866	69.8893350	31
SS_C	0	133.847884	66.8982922	15
	1	136.054881	75.9047190	16
	Total	134.986979	70.5019968	31
SS_D	0	108.498800	52.0973116	15
	1	128.016337	61.8142249	16
	Total	118.572367	57.2310995	31
SS_E	0	92.7483117	50.1608829	15
	1	126.933487	78.3900452	16
	Total	110.392273	67.4408419	31
SS_F	0	57.2224543	44.2341103	15
	1	103.246687	128.788566	16
	Total	80.9768972	98.7572467	31
SS_G	0	77.2765711	81.8788587	15
	1	101.865716	77.3877843	16
	Total	89.9677429	79.2405785	31
SS_H	0	45.9060213	40.0517953	15
	1	73.3589959	92.2752204	16
	Total	60.0752985	72.1141745	31

Table 2. The impact of exposure to novel environment (NE) and social stress (SS) on the corticosterone levels (ng/ml) of the control group (0) and SPD group (1). The means and standard deviations of the corticosterone levels were determined. The different time points, at baseline and at 15, 30, 45, 60, 90, 120 and 180 minutes after exposure, are marked as A, B, C, D, E, F, G, H respectively.

Effects of exposure to novel environment

There was no statistically linear significant interaction between the effects of play deprivation and plasma corticosterone levels after exposure to a novel environment; $F(1,27)=0.105$, $p=0.749$ (Figure 3). However, the various time points during the corticosterone response were significant over time; $F(1,27)=33.43$, $p=<0.001$. (Figure 4). Bonferroni post-hoc pairwise comparisons were performed to establish significant effects at the $p<0.05$ level. The mean scores for the baseline corticosterone levels were significantly different to every other time point, except the time point at 120 minutes after exposure. The mean corticosterone level at the latest time point, 180 minutes after exposure, was significant different to every other time point; $p<0.001$ (Table 3).

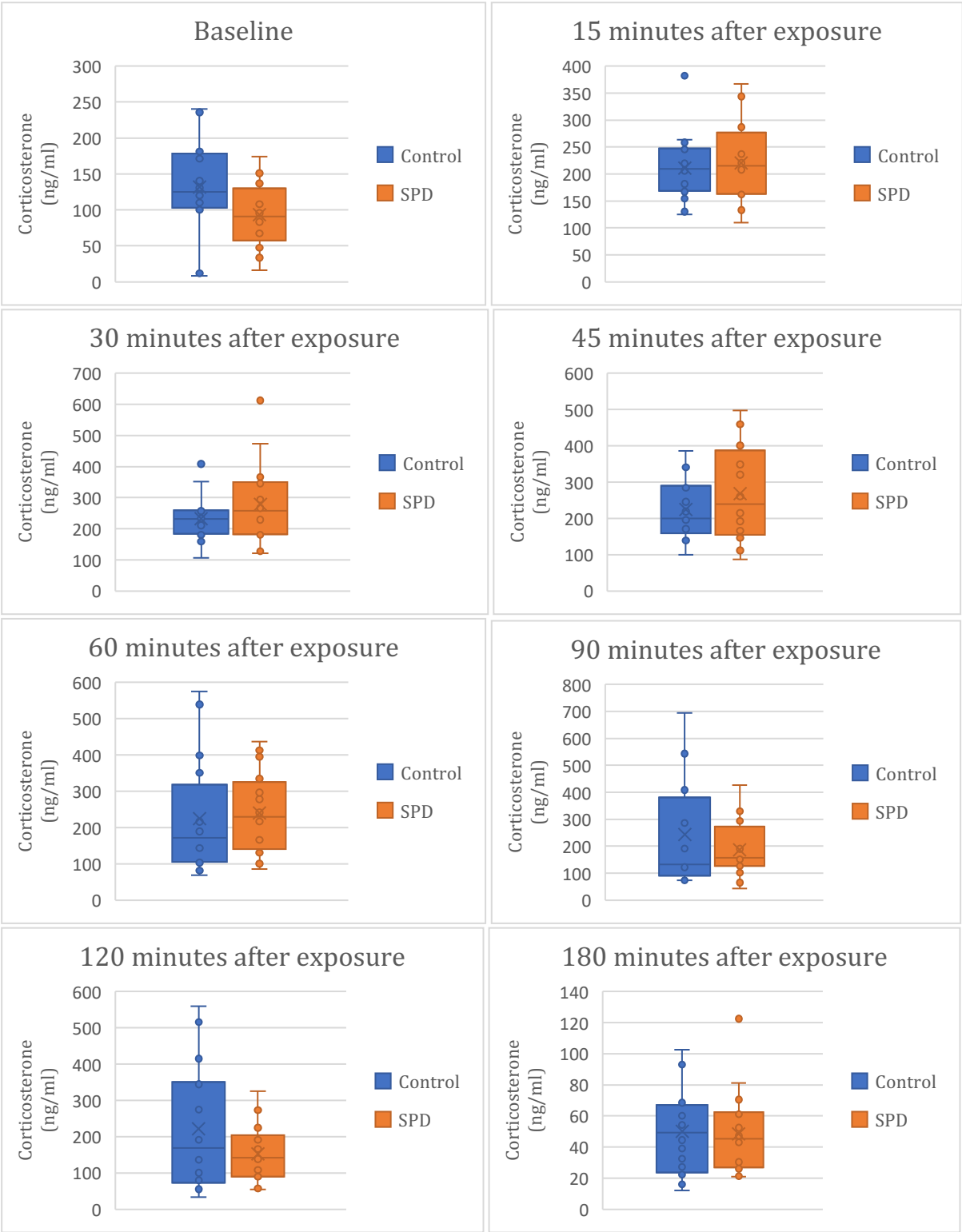

Figure 3. Box plots of the plasma corticosterone levels in control and SPD rats before the stress onset, and at 15, 30, 45, 60, 90, 120 and 180 minutes after exposure to a novel environment.

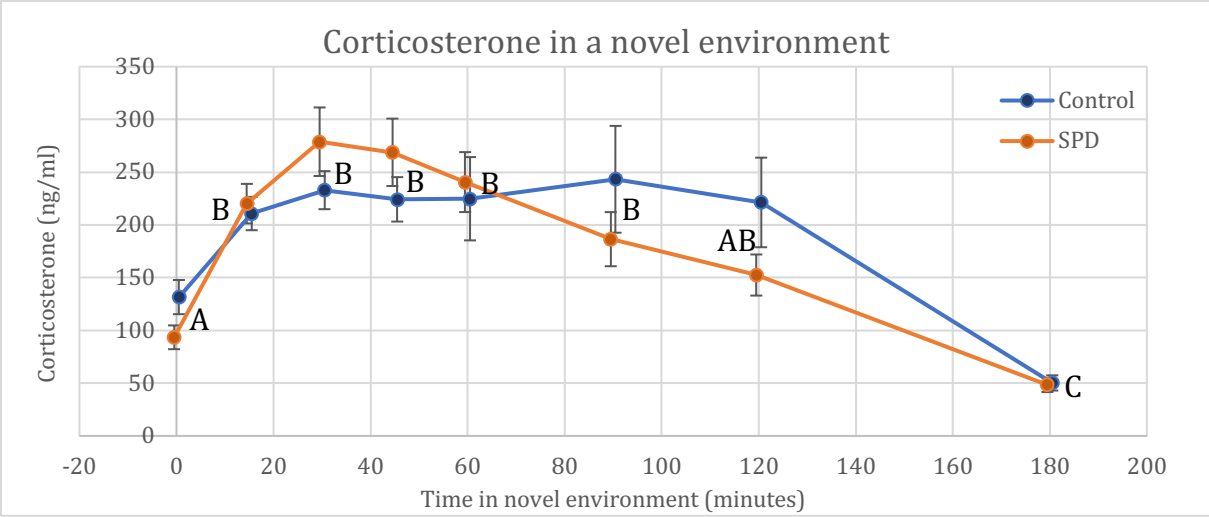

Figure 4. Corticosterone curve in a novel environment in control and SPD rats with error bars that show the 95% confidence interval. Same letters indicate no significance difference between these time points.

(I) time	(J) time	Mean Difference (I–J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-104.262*	12.562	<.001	-147.793	-60.730
	3	-144.466*	19.292	<.001	-211.320	-77.612
	4	-136.550*	19.856	<.001	-205.358	-67.742
	5	-121.100*	23.431	<.001	-202.297	-39.904
	6	-103.182*	25.831	.013	-192.695	-13.668
	7	-75.966	23.158	.080	-156.214	4.282
	8	62.278*	10.238	<.001	26.801	97.754
8	1	-62.278*	10.238	<.001	-97.754	-26.801
	2	-166.539*	11.428	<.001	-206.139	-126.939
	3	-206.744*	18.064	<.001	-269.342	-144.147
	4	-198.828*	17.683	<.001	-260.104	-137.551
	5	-183.378*	20.131	<.001	-253.138	-113.619
	6	-165.460*	23.071	<.001	-245.408	-85.511
	7	-138.244*	18.390	<.001	-201.969	-74.519

Table 3. Significant effects for the first and latest time points of the corticosterone response (ng/ml) in rats with 95% confidence interval. The means and standard deviations of the corticosterone levels were determined. The type of group was not included.

Effects of exposure to social stress

In general, there was no statistically significant interaction between the effects of play deprivation on plasma corticosterone levels after exposure to social stress; $F(1,26)=2.475$, $p=0.128$ (Figure 5).

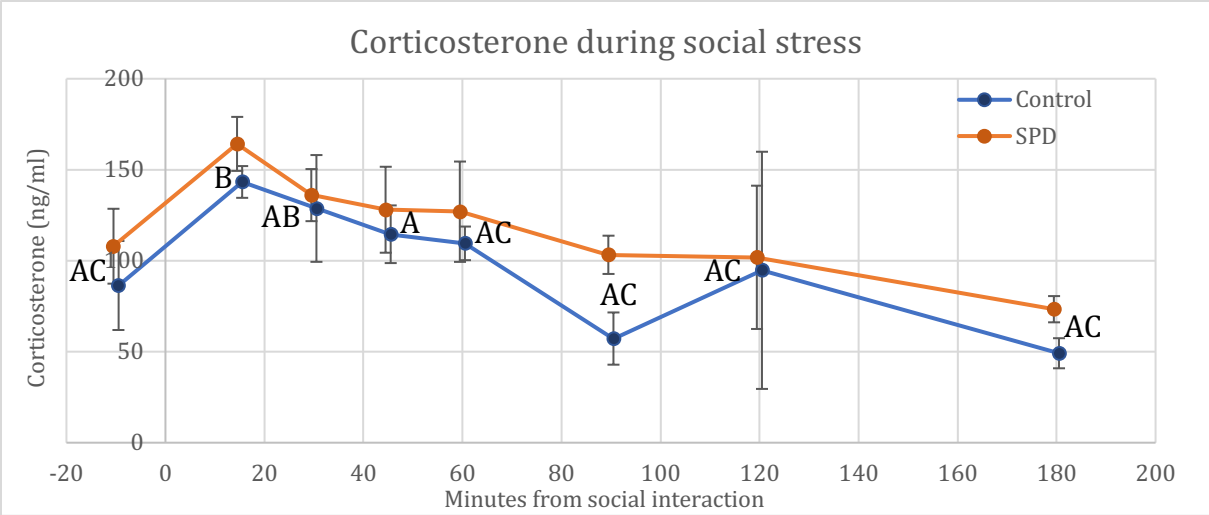

Figure 5. Corticosterone curve after exposure to social stress in control and SPD rats with error bars that show the 95% confidence interval. Same letters indicate no significance difference between these time points.

However, there was a significant effect of time on the corticosterone response; F(1,26)=30.36, p=<0.001. Bonferroni post-hoc pairwise comparisons were again performed to establish significant effects at the p<0.05 level among the various time points of the corticosterone response (Table 4). A significant difference was indicated between the second time point and every other time point, except the third time point. In addition, the mean scores for the latest time point after social stress were also significant different than the second, third and fourth time point. The result was most considerable between the second and latest time point; p<0.001.

(I) time	(J) time	Mean Difference (I–J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
2	1	57.443*	12.721	.003	13.178	101.708
	3	20.298	9.313	1.000	−12.109	52.705
	4	36.999*	7.622	.001	10.475	63.523
	5	45.439*	12.517	.034	1.884	88.994
	6	75.098*	18.890	.014	9.366	140.831
	7	65.743*	18.012	.032	3.065	128.420
	8	95.672*	16.545	<.001	38.099	153.245
8	1	−38.230	16.757	.867	−96.539	20.080
	2	−95.672*	16.545	<.001	−153.245	−38.099
	3	−75.374*	17.471	.006	−136.171	−14.578
	4	−58.673*	15.354	.021	−112.102	−5.244
	5	−50.233	19.302	.422	−117.399	16.933
	6	−20.574	22.693	1.000	−99.540	58.392
	7	−29.930	15.970	1.000	−85.501	25.641

Table 4. Significant effects for the second and latest time points of the corticosterone response (ng/ml) in rats with 95% confidence interval. The means and standard deviations of the corticosterone levels were determined. The type of group was not included.

The average corticosterone levels ranged between 5.77 and 414.87 ng mL^{-1} (mean ± SD: 120.4 ± 86.1) for the rats that had at least one clinch attack with the resident (n = 25). For the rats that did not fight with the resident (n = 7), the corticosterone levels ranged between 6.54 and 292.38 ng mL^{-1} (mean ± SD: 71.1 ± 51.9).

An independent samples t-test was conducted to compare the corticosterone levels in the rats that fought with the resident and the rats that did not fight. There was a significant difference in the scores; t=-3,099, df=13, p=0.008. When only focused on the corticosterone levels right after social stress, the independent samples t-test indicated a stronger significant difference in the scores; t=-5,248, df=19, p<0.001 (Figure 6). The type of group was not included in this test.

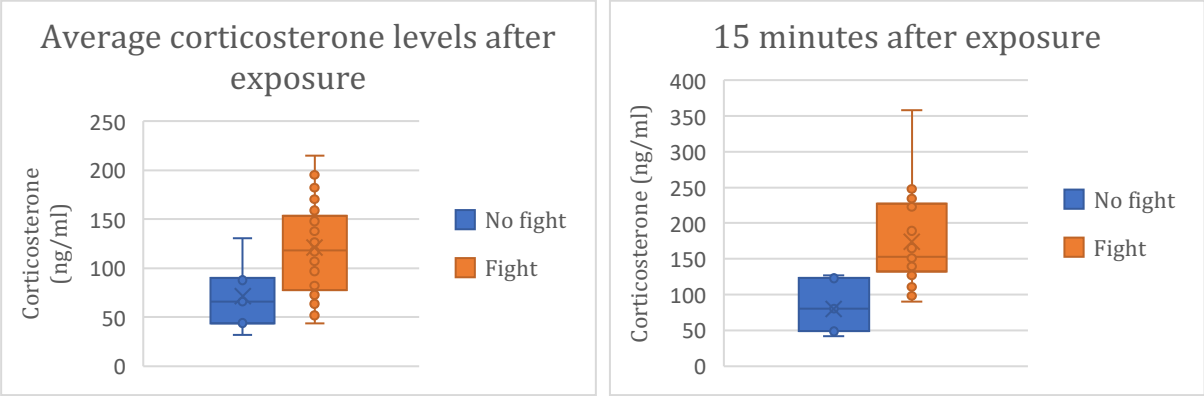

Figure 6. Box plots of the average plasma corticosterone levels and of 15 minutes after exposure to social stress in fighting and non-fighting rats. The type of group was not included.

An independent samples t-test was also conducted to compare the corticosterone levels in the fighting rats from the control group (n = 12) with the fighting rats from the SPD group (n = 13). Subsequently, the same test was carried out for the rats that did not fight from the control group (n = 4) and SPD group (n = 3). For both cases, there was no significant difference in the scores (Figure 7,8).

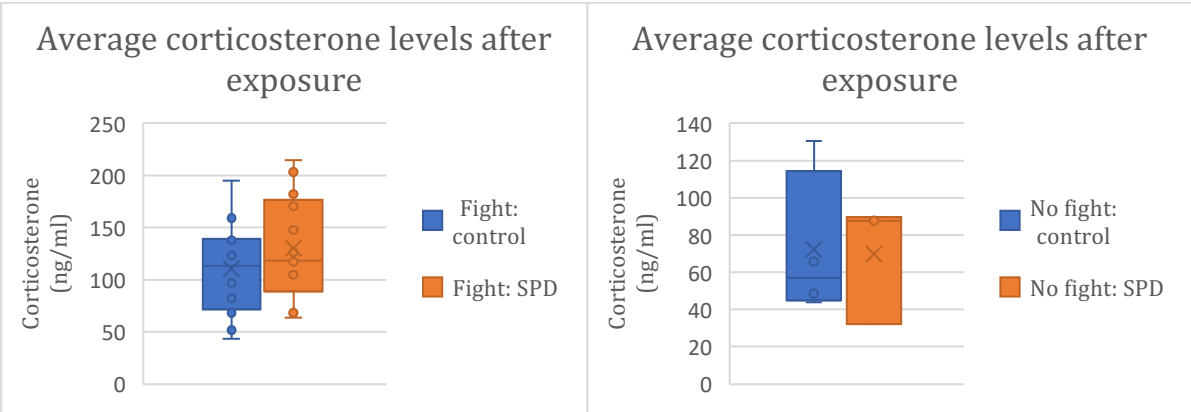

Figure 7. Box plots of the average plasma corticosterone levels at 15, 30, 45, 60, 90, 120 and 180 minutes after exposure to social stress for the fighting and non-fighting rats from the control and SPD group.

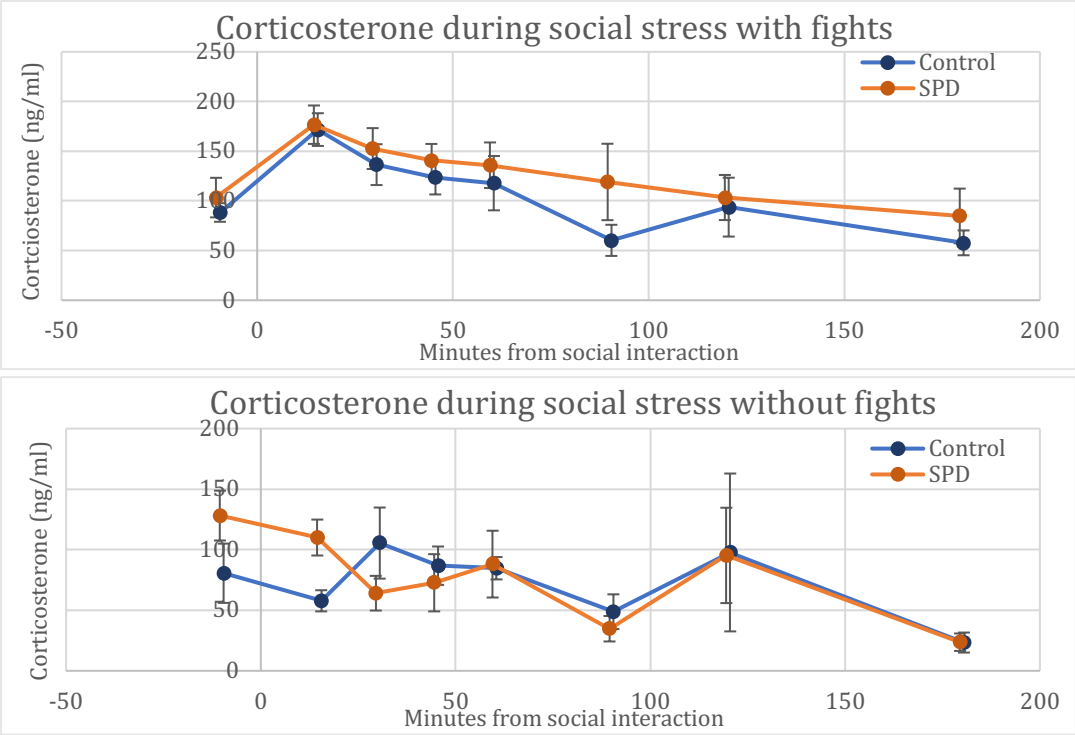

Figure 8. Corticosterone curve after exposure to social stress in control and SPD rats, with and without fights. Error bars show the 95% confidence interval. Same letters indicate no significance difference between these time points.

The behavioural data of the second stress test were also analysed with independent samples t-tests. There was no significant interaction between the effects of play deprivation on behaviour after exposure to social stress.

The total duration of social exploration from the intruder ranged between 0.00 and 88.96 s (mean ± SD: 37.3 ± 27.3) for the rats that had at least one clinch attack with the resident. For the rats that did not fight with the resident, the duration of social exploration ranged between 43.36 and 192.42 s (mean ± SD: 103.5 ± 45.1).
An independent samples t-test was conducted to compare the duration of social exploration in the rats that fought with the resident and the rats that did not fight. There was a significant difference in the scores; t=3.702, df=7, p=0.008 (Figure 9). The type of group was not included in this test.

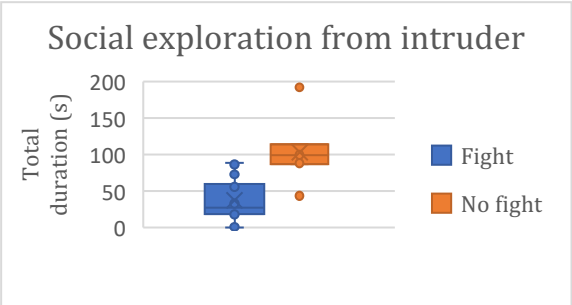

Figure 9. Box plot of the total duration of social exploration from the intruder in fighting and non-fighting rats.

A positive correlation was found between the amount of clinch attacks and the total freezing time of the SPD group; r=0.676, p=0.004. Nonetheless, this correlation was not significant for the control group; r=0.443, p=0.086. The amount of clinch attacks was found to be negatively correlated with the total duration of social exploration from intruders of the control group; r=-0.688, p=0.003, and intruders of the SPD group; r=-0.648, p=0.007 (Figure 10).

A moderately positively Pearson's correlation was found in the total amount of clinch attacks and the increase of the plasma corticosterone level between baseline and 15 minutes after exposure to social stress; r=0.593, p=0.016 for the control group and r=0.532, p=0.034 for the SPD group.

The increase of the plasma corticosterone level between baseline and at 15 minutes after exposure to social stress was also found to be moderately positively correlated with a few behavioural acts for the SPD group, including the total freezing time; r=0.563, p=0.023, and the total amount of submissive posture; r=0.500, p=0.049. This correlation was not significant for control rats.

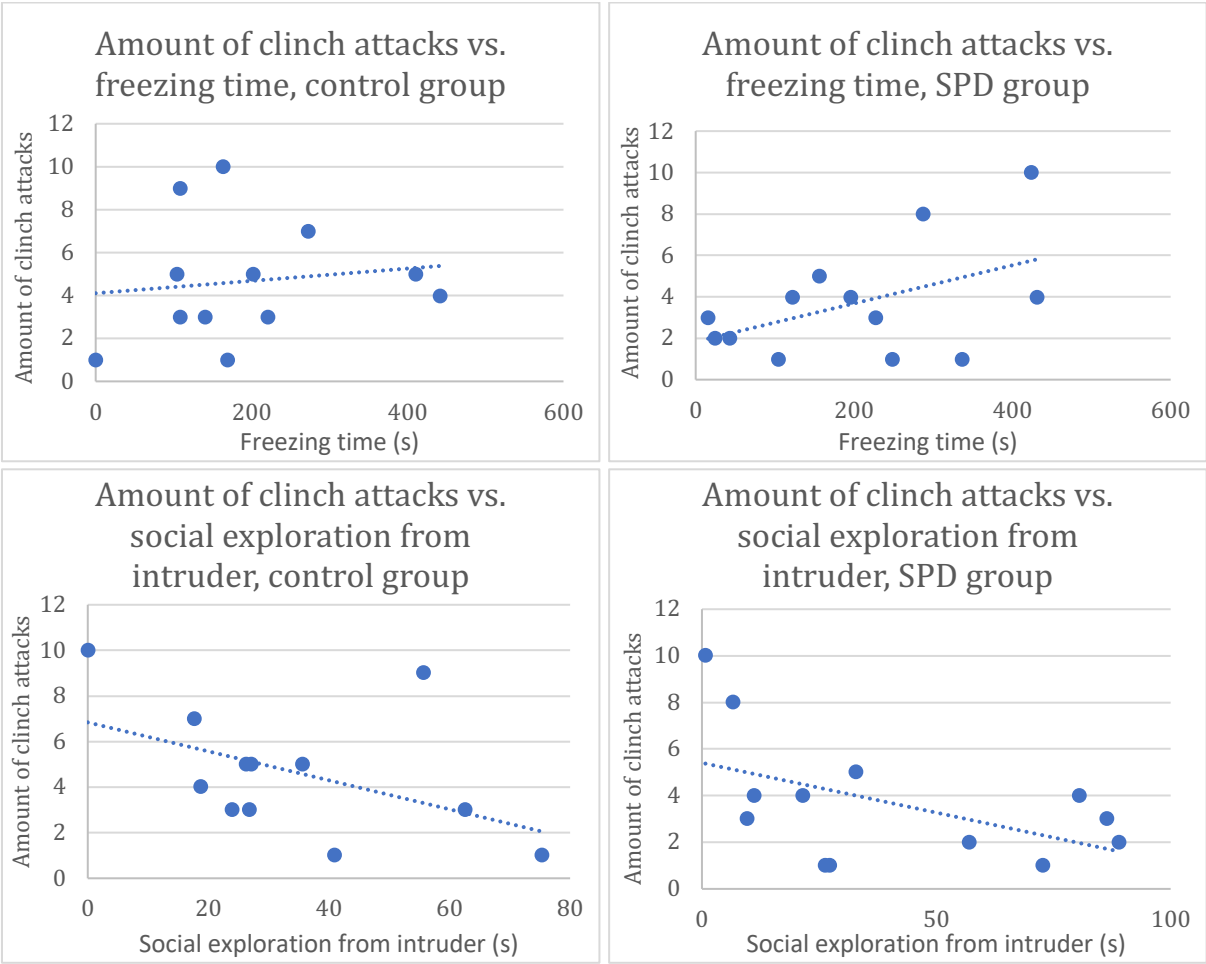

Figure 10. Correlations in behaviour after exposure to social stress.

For both the residents and intruders, the behavioural acts and postures were scored and the mean and standard deviation of the outcomes were calculated (Table 5,6).

Intruder: Control group	*Moving towards*	*Social exploration*	*Non-social exploration*	*Freezing*	*Upright posture*	*Clinch attack*	*Submission*
Mean of total duration (s)	9,34	47,13	350,03	146,22	22,41	3,46	20,62
SD of total duration (s)	2,40	8,10	35,74	35,00	5,40	0,86	11,46
Mean of total number	4,1	13,3	23,8	11,2	3,8	3,5	1,9
SD of total number	1,0	1,8	2,1	2,9	0,9	0,8	0,5
Intruder: SPD group	*Moving towards*	*Social exploration*	*Non-social exploration*	*Freezing*	*Upright posture*	*Clinch attack*	*Submission*
Mean of total duration (s)	6,82	56,43	329,33	163,77	27,99	2,71	11,98
SD of total duration (s)	2,03	12,51	36,03	37,57	8,38	0,62	5,80
Mean of total number	2,8	12,8	24,7	13,6	4,1	3,0	1,7
SD of total number	0,8	2,5	2,9	3,3	1,0	0,7	0,5

Table 5. Mean and standard deviation of the different behavioural acts and postures of the intruders.

Resident: Control group	*Moving towards*	*Ano-genital sniffing*	*Social exploration*	*Non-social exploration*	*Inactivity*	*Upright posture*	*Lateral threat*	*Clinch attack*	*Keep down*
Mean of total duration (s)	3,60	50,37	111,29	355,82	54,94	3,40	8,29	3,55	8,23
SD of total duration (s)	1,28	8,43	12,41	16,66	15,30	1,18	2,00	1,14	3,47
Mean of total number	2,1	10,2	30,2	29,5	7,3	2,6	7,6	2,6	1,7
SD of total number	0,6	1,6	3,0	1,3	2,0	0,9	1,6	0,6	0,6
Resident: SPD group	*Moving towards*	*Ano-genital sniffing*	*Social exploration*	*Non-social exploration*	*Inactivity*	*Upright posture*	*Lateral threat*	*Clinch attack*	*Keep down*
Mean of total duration (s)	3,82	45,46	114,96	346,66	68,46	4,11	8,21	2,66	5,04
SD of total duration (s)	1,78	8,98	10,80	20,49	23,51	1,25	1,86	0,67	1,62
Mean of total number	1,8	9,6	29,6	28,5	5,9	2,6	7,4	2,3	1,1
SD of total number	0,5	1,7	2,6	1,9	1,2	0,7	1,5	0,6	0,3

Table 6. Mean and standard deviation of the different behavioural acts and postures of the residents.

Discussion

The aim of the present study was to investigate the relation between social play behaviour and stress responsivity. To that end, a total of two stress tests were performed with two groups of rats, involving a group of rats that were deprived from social play (SPD) at two weeks of age for three weeks and a group of control rats that were not isolated during early development. In the first stress test, rats were exposed to a novel environment. In the second stress test, the rats were exposed to social stress using the resident intruder protocol. Consequently, the behavioural data and corticosterone response to these stressful stimuli were determined of the two different groups and were compared against each other.

Despite no statistically linear significant interaction being found between the effects of play deprivation on plasma corticosterone levels in both stress tests, there were significant differences among the various time points of the corticosterone response.

In the first stress test, the corticosterone levels had peaked at 30 minutes after exposure to a novel environment for the SPD group, and at 90 minutes after exposure for the control group. In the second stress test, the corticosterone levels had peaked at 15 minutes after exposure to social stress for both the SPD and control group. However, in both stress tests, the largest increase in corticosterone levels occurred 15 minutes after exposure to the stressful stimuli. Subsequently, the corticosterone curve slowly declined over time, until the same levels as baseline had been reached again at 180 minutes after exposure. Moreover, these stress tests demonstrate the fast corticosterone response to acute stress, followed by an extensive time interval that is needed for the corticosterone levels to be restored.

A comparison was also made between the corticosterone response to social stress of rats that had at least one clinch attack with the resident and rats that did not fight with the resident at all. It revealed significantly higher corticosterone levels in the fighting rats, with their corticosterone curve resembling the general corticosterone curve of the second stress test. The largest increase in corticosterone levels occurred 15 minutes after exposure to social stress, along with the corticosterone curve slowly declining over time until the same levels as baseline had been reached again at 180 minutes after exposure. Contrarily, these characteristics were not featured in the corticosterone curve in the non-fighting rats. Especially considering that there was no increase in corticosterone levels right after exposure to social stress, but without any fight with the resident, it is conceivable that clinch attacks trigger a fast corticosterone response and are therefore, acknowledged as stressful stimuli. Besides, the non-fighting rats spent significantly more time in social exploration than fighting rats.

Despite no significant interaction being found between the effects of play deprivation on behaviour after exposure to social stress, there were some interesting correlations.

The positive correlations of the SPD group between the increase of the plasma corticosterone level within 15 minutes after exposure to social stress and different behavioural acts, involving the total freezing time and the total amount of submissive posture, may indicate that social play deprivation causes a higher sensitivity of the HPA axis as these correlations were not significant for the control group. This interpretation could also be supported by the strong positive correlation between the amount of clinch attacks and the total freezing time for only the SPD group, and may be a motive for further investigation.

Furthermore, there was a statistically significant interaction between the effects of the particular experiment day on plasma corticosterone levels after exposure to a novel environment; $F(1, 27)=5.024$, $p=0.033$ (Figure 11). However, there was not a statistically significant interaction between the effects of the particular experiment day on plasma corticosterone levels after exposure to social stress; $F(1,26)=0.403$, $p=0.531$. According to earlier studies, the consistency of increased corticosterone levels after stress depends on the type of stressor used, conditions of the separation procedure, and the age of testing (Rees, Steiner, & Fleming, 2006). This suggests that corticosterone levels have a remarkable sensitivity and could be easily affected.

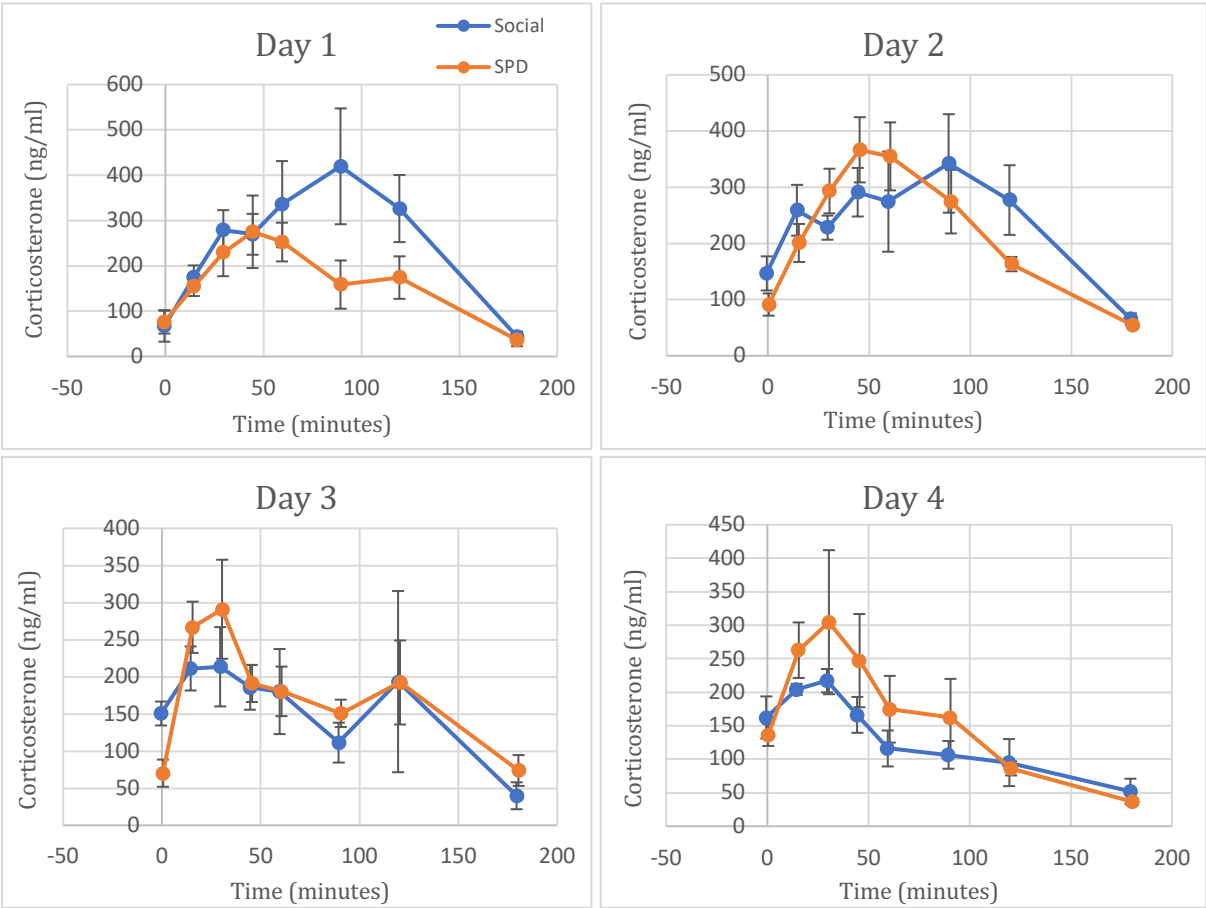

Figure 11. Corticosterone curve in a novel environment on the different experiment days.

The first stress test was carried out in the presence of all rats, while the second stress test was executed in a separate room. The corticosterone levels could also be affected by the witness of a stress test, as it may trigger a physiological stress response. Nonetheless, this was not taken into consideration in the stress tests and cannot be determined from this study, though it would be interesting to gain more insight into the impact of environmental factors. Also, the recovery period of the corticosterone response after exposure to social stress seemed to be shorter than after exposure to a novel environment. This suggests a sensitivity of the corticosterone response to different stressors. It is recommended to do more research on this sensitivity, so light can be shed on the corticosterone response and its recovery time for a specific stressor. As a result, the relation between social play deprivation and stress responsivity would presumably be more accessible and reliable to study.

Conclusion

In the present study, there was no evidence presented to support the notion that SPD rats have a different responsivity to stress compared to control rats. However, the increase of the plasma corticosterone level between baseline and at 15 minutes after exposure to social stress was found to be moderately positively correlated with a few behavioural acts for the SPD group, including the total freezing time and the total amount of submissive posture, but not for the control group. A positive correlation was also found between the amount of clinch attacks and the total freezing time of the SPD group, though this correlation was not significant for the control group. This may indicate that social play deprivation causes a higher sensitivity of the HPA axis.

Furthermore, there were significant differences among the various time points of the corticosterone response in both stress tests. There was also a statistically significant interaction between the effects of the experiment day on plasma corticosterone levels after exposure to a novel environment. This demonstrates the dynamics of the corticosterone response and suggests a sensitivity of corticosterone to stressors and environmental factors.

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