Methamphetamine exposure during the first, but not the second half of prenatal development, affects social play behavior

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Summary

Methamphetamine (MA), as a psychostimulant drug that crosses the placental barrier, may disrupt the development of social play. The present study aims to examine the effect of prenatal MA (5 mg/kg) exposure during the first (gestational day (GD) 1-11) or second (GD 12–22) halves of prenatal development of rats on social play behavior. To investigate an acute effect of MA on social play in adulthood, juvenile rats were exposed to a dose of 1 mg/kg MA or saline on the test day and tested for social play for 15 min. Prenatal exposure to MA during GD 1–11 increased social play behavior during 5-10 min interval of the test in males but not females. Prenatal MA during GD 12–22 did not influence social play in males nor females. However, social play occurred to a greater extent in GD 12–22 groups compared with GD 1–11. Acute exposure to MA eliminated playful behavior in all groups and decreased social exploration in GD 1–11. Our results suggest that manipulation of prenatal development during the first half of the gestational period has a greater impact on social play behavior than during the second half.

Introduction

An essential part of the development, which is seen in all mammalian species, involves a characteristic form of rough-and-tumble play known as social play behavior (Panksepp *et al.* 1984; Rubin *et al.* 1978; Špinka *et al.* 2001). It is the earliest form of social behavior directed toward peers and not the mother (Pellis and Pellis 1998; Vanderschuren *et al.* 1997). Social isolation during the time when social play reaches its peak leads to long-lasting disturbances in emotional, social, and cognitive skills (Van den Berg *et al.* 1999; Vanderschuren and Trezza 2014). Neurodevelopmental disorders show similar behavioral abnormalities (Jordan 2003; Nijhof *et al.* 2018; Ryan *et al.* 2018). Social play is modulated by neurotransmitter systems involved in reward and motivation circuits and is as highly sought as food, sex, and drugs of abuse, which makes social play a natural reinforcer (Douglas *et al.* 2004; Siviy and Panksepp 2011; Vanderschuren *et al.* 1997).

A euphoric state, increased energy, and heightened vigilance are induced not only by social play but also by amphetamines, which are synthetic psychostimulant drugs. These effects are mediated by increasing the synaptic concentration of the neurotransmitters dopamine (DA), noradrenaline (NA), and to a lesser extent serotonin (5-HT), by stimulating their release and inhibiting their reuptake (Rothman and Baumann 2003; Rothman *et al.* 2001; Sulzer *et al.* 1995). Psychostimulants such as methylphenidate, amphetamine, and cocaine have been shown to suppress social play (Siviy and Panksepp 2011; Trezza *et al.* 2010). It seems that the effect of cocaine is mediated, in part, through dopaminergic neurotransmission, whereas the effects of amphetamine and methylphenidate are mediated through NA (Achterberg *et al.* 2014; Trezza *et al.* 2010; Vanderschuren *et al.* 2008). DA plays an important role in motivating social play, whereas NA influences its emotional and cognitive aspects. However, the effect of methamphetamine (MA) on social play behavior has not been addressed in the literature, and there is a lack of research on the prenatal effects of psychostimulants on social play behavior.

The research of our laboratory is focused on the prenatal effect of MA. Due to its lipophilic character, MA crosses the placenta when abused during pregnancy (Burchfield *et al.* 1991; Dattel 1990) and enters breast milk during lactation (Rambousek *et al.* 2014). These represent mechanisms by which MA can impair both the prenatal and postnatal development of the fetus. Our recent studies have shown that prenatal exposure to MA impairs postnatal sensorimotor development of rat pups (Malinová-Ševčíková *et al.* 2014; Šlamberová *et al.* 2006). Furthermore, the adverse effects of MA can affect the second generation of rats, which were

not exposed to MA (Šlamberová *et al.* 2007). Prenatal exposure to MA impairs cognition (Hrebíčková *et al.* 2016; Macúchová *et al.* 2017; **Šlamberová** *et al.* 2014), affects the stress response (Holubová *et al.* 2017), anxiety (Macúchová *et al.* 2016), and pain sensitivity later in life (Yamamotová *et al.* 2011). The exposure to MA during prenatal development may sensitizes rats to the same or various drugs in adulthood (Macúchová and Šlamberová 2017). The present study is the first of our studies to examine the effect of MA on social play behavior of juvenile rats. However, in our previous studies, rats prenatally or neonatally exposed to various psychostimulants were observed as adults to assess their social interactions (social interaction test SIT). The studies demonstrated that psychostimulants, such as MA, amphetamine, cocaine, and 3,4-methylenedioxymethapmhetamine (MDMA) suppress social interaction in adult male rats in a dose-specific manner (Šlamberová *et al.* 2015a; Šlamberová *et al.* 2010). Social interactions in adulthood were decreased in rats prenatally exposed to MA in the same periods like the present study (Hrebíčková *et al.* 2017). Therefore, we hypothesized that MA would also cause changes in social play behavior during adolescence.

In line with our previous research (Hrebíčková *et al.* 2014; Hrebíčková *et al.* 2017; Hrebíčková *et al.* 2016; Malinová-Ševčíková *et al.* 2014; Ševčíková *et al.* 2017), the present study continues to examine the effects of MA during different developmental stages, i.e., the first or second half of gestation. It has been shown that different brain structures mature during different developmental stages (Bayer *et al.* 1993). Neurulation, a process that occurs early in embryogenesis, is completed by approximately gestational day (GD) 11 (Rice and Barone 2000). Moreover, teratogenic insults are more likely to occur during early organogenesis, which in rats is between GD 7–12 (Schmidt and Johnson 1997). Regional development of the brain continues into the postnatal period. The prefrontal cortex, which plays a key role in the cognitive part of social play behavior, develops from GD 12 to postnatal day (PD) 20 (Kolb *et al.* 2012). The neurons of the mesocorticolimbic DA system, which are the structural base for the emotional processes of social play, are seen, for the first time, on GD 13 (Rice and Barone 2000). Neurons of the noradrenergic system, which are assumed to be involved in the arousal response, attention, anxiety, and learning and memory (Herlenius and Lagercrantz 2004), appear between GD 12–14 (Sundstrom *et al.* 1993). MA would seem to have the potential to influence those brain regions that are developing at the time of drug exposure.

Based on the previous findings, the present study aimed to investigate whether the frequency and duration of social play behavior elements changed in response to prenatal MA exposure. Second, we investigated the acute effect of MA exposure in adolescence on social play

behavior. We hypothesized that MA would have an adverse effect on the offspring in both the early and late prenatal exposure groups. However, we expected the effects of MA during the first half of prenatal development, based on the demanding processes of embryogenesis, to have a greater effect. Moreover, an acute dose of MA was also expected to impair social play behavior.

Materials and Methods

Prenatal animal care and prenatal drug treatment

Adult albino Wistar rats, raised by Charles River Laboratories International, Inc. and delivered by Velaz (Prague, Czech Republic) were used. Males (300–350 g) were housed 4 per cage and females (250–300 g) were housed 5 per cage. Rats were kept in a temperature-controlled (22–24 °C) colony room, on a 12:12 h light: dark cycle with lights on from 0600 h, with free access to food and water. After one week of acclimation, females were smeared (vaginal lavage) to determine their phase of the estrous cycle. At the onset of the estrous phase, one female and one male were housed overnight. The next morning females were smeared again for the presence of sperm and that day was counted as the first day of gestation (GD 1). Females were randomly assigned to those, who would receive MA or saline (SA) during GD 1–11 and those, who would receive MA or SA during GD 12–22. For 11 days of gravidity, mothers were subcutaneously injected (s.c.) with MA at 5 mg/kg/day (volume 1 ml/kg/day). A dose of 5 mg/kg of MA has been shown to induce comparable fetal brain drug concentrations and similar behavioral changes to those found in humans (Acuff-Smith *et al.* 1992). SA females were injected with s.c. saline at the same time and the same volume (1 ml/kg/day) as the MA group. The day of delivery was designated as PD 0. Mothers and their litters were left undisturbed in their cage on that day.

Litter characteristics

On PD 1, pups born on the same day were cross-fostered, and litter size was adjusted to 14. Whenever possible, the same numbers of MA and SA-exposed pups, as well as male and female pups, were kept in each litter. For identification, the pups were tattooed on the left or right leg to indicate prenatal MA or SA exposure, respectively. On PD 21, the pups were weaned and split into two cages according to sex. Pups were left in these cages until PD 28.

Social play behavior and acute drug treatment

Behavioral procedures of social play used in the present study were mostly reproduced according to studies by Vanderschuren, Achtenberg, and Trezza (e.g., (Achterberg *et al.* 2014; Trezza *et al.* 2009a; Vanderschuren *et al.* 2008)). Two weeks before testing, the light-dark cycle was reversed in the colony room (lights on for 12 h, from 1800 h). The experiments were performed in a plexiglas cage measuring 43×26.5×18 cm (l ×w ×h), with wood shavings covering the floor, in a room under dim light conditions. On PD 28 and 29, each animal was individually habituated to the test cage for 10 min. To facilitate the expression of social play behavior during testing, rats were socially isolated the whole night before testing (approximately 16 hours). On the test day, PD 30, pairs of rats were exposed to an acute dose of MA (1 mg/kg) or SA at the same volume (1 ml/kg) 45 minutes before testing. The plasma and brain concentrations, at a dose of 1 mg/kg of MA, have been shown to peak 45–60 min after exposure (Rambousek *et al.* 2014). According to our preliminary and published data (Schutová *et al.* 2010), a 1 mg/kg dose of MA does not induce stereotypical behavior in control adult rats.

For the test, two rats with the same gestation exposure period, sex, prenatal, and acute drug exposure were placed into the test cage and recorded for 15 min. One pair of rats were considered as one unit. To reduce the influence of dominance, rats were paired with other rats with a very similar body weight and with rats that were not cage mates. A video camera was used for recording, and the recordings were analyzed later. The recordings were examined using the ODLog (Macropod Software) program. The frequency of pinning (i.e., one of the animals laying in supine position on the floor with the other animal standing over it, which serves as a social releaser of a continuing play round) and the frequency of pouncing (i.e., one of animals soliciting the partner by attempting to nose or rub the nape of its neck) were scored as elements of social play behavior. Pinning and pouncing are considered to be the major elements for social play behavior in rats, because they are simulated by social isolation and alternate with other playful elements such as boxing or following (Vanderschuren *et al.* 2016; Vanderschuren *et al.* 1997). Also, the overall duration of social play behavior (i.e., pinning, pouncing, boxing – struggling using the forepaws in vertical position) was measured as well as the duration of non-playful forms of social interactions (i.e., climbing over the partner, sniffing or grooming the partner). The rest of the 15 min, during which rats did not play or explore each other, represent other activities, such as space exploration (sniffing, rearing, and manipulating shavings), self-grooming or freezing. Other activities were not measured, since they do not refer to social play behavior (Vanderschuren *et al.* 1997). For a more detailed

explanation of the social play behavior elements see the studies by Vanderschuren, Trezza or Achterberg (e.g., (Achterberg *et al.* 2014; Trezza *et al.* 2009a; Vanderschuren *et al.* 1997)).

Statistical analysis of social play behavior

Eight pairs of pups were used in each group. Frequencies of pinning and pouncing, total play duration, and duration of social exploration were calculated per pair and expressed as a mean ± SEM. To determine whether the prenatal and acute effects of MA on social play behavior changed over time, the 15-min test session was divided into three 5-min intervals, which were analyzed using a three-way ANOVA with repeated measures (*prenatal drug × acute drug × time*). A one-way ANOVA (*acute drug)* was used to analyze the single acute effect of MA. To assess the differences among the prenatal exposure periods, a one-way ANOVA *(exposure period)* was used. Analyses were followed by the Fisher´s LSD post-hoc test where appropriate. Differences were considered significant if p < 0.05. STATISTICA software was used for statistical analyses.

Results

For a clearer illustration of overall rats behavior, proportion of social play duration, social exploration, and other non-social activities during 15 min of the social play test is displayed in Figure 1. Play duration includes pouncing, pinning and boxing. Social exploration represents sniffing and grooming the partner or climbing over the partner. Other activities include space exploration (sniffing, rearing, and manipulating shavings), self-grooming or freezing.

Effect of prenatal and acute MA exposure on social play behavior

To determine whether the effects of prenatal MA on social play behavior changed over time, the 15-min test session was divided into three 5-min intervals. The three-way ANOVA with repeated measures (*prenatal drug × acute drug × time*) revealed differences in a group of males of GD 1–11 exposure period. Pinning [F(2,56) = 6.05; p = 0.004**] (Figure 2A) and pouncing [F(2,56) = 5.26; p = 0.008**] (Figure 2B) increased during the middle time interval (i.e., 5–10 min) in males prenatally but not acutely exposed to MA. Total play duration increased during the middle time interval but decreased during the last time interval (i.e., 10–15 min) of the test [F(2,56) = 9.07; p = 0.0004***] (Figure 2C). The exposure to prenatal MA did not affect social exploration in any of the intervals [F(2,56) = 1.34; p = 0.27] (Figure 2D). The effect of acute MA was significant in pinning, pouncing and total play duration and is

mentioned in the paragraph below. There were no significant effects in social play behavior for other groups, i.e., females of GD 1–11 exposure period, males and females of GD 12–22 exposure period. The resultas are as follows: group of females of GD 1–11 exposure period – pinning $[F(2, 56) = 0.80; p = 0.46]$, pouncing $[F(2, 56) = 0.71; p = 0.49]$, total play duration $[F(2, 56) = 0.21; p = 0.81]$, and social exploration $[F(2, 56) = 0.15; p = 0.86]$; males of GD 12–22 exposure period – pinning $[F(2, 56) = 0.002; p = 0.99]$, pouncing $[F(2, 56) = 0.33; p = 0.72]$, total play duration $[F(2, 56) = 0.75; p = 0.48]$, and social exploration $[F(2, 56) = 1.96; p = 0.15]$; females of GD 12–22 exposure period – pinning $[F(2, 56) = 0.50; p = 0.61]$, pouncing $[F(2, 56) = 1.47; p = 0.24]$, total play duration $[F(2, 56) = 2.21; p = 0.12]$, and social exploration $[F(2, 56) = 2.45; p = 0.09]$. For the statistical analyses, groups of males and females were used separately.

There was a main effect of acute drug exposure: the one-way ANOVA *(acute drug) showed that* an acute dose of MA (1 mg/kg) eliminated social play behavior. Acute MA exposure decreased pinning in GD 1–11 exposure period $[F(1,56) = 71.37; p < 0.001]$; GD 12–22 exposure period $[F(1,56) = 199.65; p < 0.001]$ (Figure 3A); pouncing in GD 1–11 exposure period $[F(1,56) = 109.28; p < 0.001]$; GD 12–22 exposure period $[F(1,56) = 249.43; p < 0.001]$ (Figure 3B); total play duration in GD 1–11 exposure period $[F(1,56) = 89.67; p < 0.001]$, GD 12–22 exposure period $[F(1,56) = 259.55; p < 0.001]$ (Figure 3C). Social exploration was decreased by an acute dose of MA in the GD 1–11 rats $[F(1,56) = 13.14; p < 0.001]$, but unchanged in the GD 12–22 rats $[F(1,56) = 2.41; p = 0.13]$ (Figure 3D).

Effect of prenatal exposure periods on social play behavior

There was a main effect of prenatal exposure periods: application within GD 12–22 (regardless of the drug exposure) increased the rats pinned $[F(1,60) = 12.19; p < 0.001]$ (Figure 4A) and pounced $[F(1,60) = 11.13; p < 0.01]$ (Figure 4B) as well as total play duration $[F(1,60) = 6.46; p < 0.05]$ (Figure 4C) relative to exposure within GD 1–11 exposure period. On the other hand the social exploration was increased $[F(1,60) = 35.26; p < 0.001]$ in rats administered within GD 1–11 than GD 12–22 (Figure 4D).

Discussion

Investigating the effects of drugs of abuse on social play behavior helps us to answer two questions. First, what are the neural substrates of social play behavior and second, how do drugs of abuse affect the development of social interactions prenatally and postnatally (Achterberg *et al.* 2014)? Therefore, the present study examined the effect of MA exposure

during the first and second half of prenatal development relative to social play behavior and the acute effect of MA on social play behavior in adolescence. We expected that prenatal exposure to MA would cause changes in brain structures that are developing during the time of drug exposure, which would lead to corresponding functional changes. In addition, we expected more adverse MA exposure effects during GD 1–11 compared with GD 12–22 since GD 1–11 included the demanding processes of embryogenesis (Bayer *et al.* 1993).

We found that pouncing, pinning, and total play duration were increased after prenatal but not acute MA exposure in males of GD 1–11 exposure period, but only in the middle interval of the test. Prenatal MA GD 12–22 exposure did not produce any changes in social play elements or social exploration. Additionally, acute MA exposure eliminated social play behavior. Social exploration was decreased after acute MA exposure in GD 1–11 groups regardless of prenatal treatment. All social play behavior elements were decreased in the GD 1–11 rats compared with GD 12–22 rats irrespective of the prenatal drug (i.e., MA or SA).

The first goal of the present study was to examine the prenatal effect of MA on social play behavior. Deficits or decrease in social play behavior was expected. Surprisingly, social play elements and total play duration were increased in the middle interval of the test in males prenatally but not acutely exposed to MA, what is difficult to interpret. Since there is an absolute lack of studies examining prenatal MA or amphetamine effect on social play behavior, studies referring to the prenatal effect of cocaine might be explicatory. However, it should be mentioned that the mechanism of action of MA and cocaine is mediated through different neurotransmitters (Achterberg *et al.* 2014). In a study by Wood *et al.* (1994) social play behavior was decreased after prenatal cocaine exposure (GD 8–20). In the next study by these authors (Wood *et al.* 1995), pinning and pouncing were unaffected when the adolescent rats were exposed to a stressor (foot-shock, forces swim or white noise stress), suggesting that stress might have normalized play behavior in offspring prenatally exposed to cocaine. Neugebauer *et al.* (2004) reported that environmental manipulations weaken the effects of prenatal cocaine on social play behavior. While rearing in an impoverished environment increased play solicitation in rats prenatally exposed to cocaine, those raised in an enriched environment did not differ from controls. In a study by Magalhaes *et al.* (2006), play fighting, solicitation (pouncing) and social investigation were unaffected in offspring prenatally exposed to cocaine, when reared in a standard environment. However, rearing in an enriched environment decreased both play behavior and social investigation, explained that animals

reared in an enriched environment prefer to explore a novel environment instead of engaging in social play (Sobrian and Holson 2011).

The second goal of this study was to investigate the acute effect of MA exposure, relative to social play behavior. Ours (Hrebíčková *et al.* 2017; Šlamberová *et al.* 2015a; Šlamberová *et al.* 2010) and other studies (Davidson *et al.* 2001; Syme and Syme 1974) demonstrated decreased social contact after acute MA exposure. Moreover, it has been shown that rats were still significantly less social several weeks after MA exposure than rats without MA exposure. On the SIT, prenatally MA-exposed males, when given an acute MA, amphetamine, and MDMA exposure, showed decreased time spent in social interactions compared with SA-exposed rats exposed in adulthood to the same drugs (Šlamberová *et al.* 2015b). Coming back to social play, it was shown that acute doses of cocaine, amphetamine, methylphenidate, and MDMA can all suppress social behavior at doses too low to induce psychomotor hyperactivity (Achterberg *et al.* 2014; Homberg *et al.* 2007; Vanderschuren *et al.* 2008). Our results are thus in line with other studies.

Social exploration is a standard parameter of anxiety used on the SIT (File and Seth 2003). It has been shown that playful social interactions are more rewarding than non-playful social interactions (Calcagnetti and Schechter 1992; Trezza *et al.* 2009b). In the present study, behaviors, which manifested interest in conspecific (e.g., sniffing, following, grooming) without elements of social play behavior (i.e., pouncing and pinning) was assigned as social exploration. Social exploration was decreased after acute MA exposure regardless of prenatal drug exposure in GD 1–11 rats, which is in agreement with our previous findings that social interactions in adult male rats decreased after acute exposure to MA, amphetamine, MDMA, and cocaine (Hrebíčková *et al.* 2017; Šlamberová *et al.* 2015a; Šlamberová *et al.* 2010). By contrast, neither prenatal nor acute exposure to MA produced any differences in groups prenatally exposed during GD 12–22. Moreover, no changes in social exploratory behavior and locomotor activity have been shown after the infusion of play-suppressors such as methylphenidate and atomoxetine into structures that are hypothesized to be the anatomical background of the emotional properties of social play, i.e., the anterior cingulate and infralimbic cortex, basolateral amygdala, and habenula (Achterberg *et al.* 2015).

In the present study, we have extended our previous findings from the SIT on adult rats. Nonetheless, social play behavior and social interaction in adult rats are regulated differently in the brain and may represent two different categories of behavior (Vanderschuren *et al.* 1995a; Vanderschuren *et al.* 1995b). Recently, the neurocircuitry of social play behavior that

is affected by stimulants seems to have been revealed. It was demonstrated that amphetamine and methylphenidate inhibit social play via a noradrenergic mechanism of action. Their play-suppressant effect was imitated by the NA reuptake inhibitor atomoxetine, but not the DA reuptake inhibitor GBR12909, and their effect on social play was blocked by pretreatment with an antagonist of the α2-adrenoceptor, but not a DA receptor antagonist (Achterberg *et al.* 2014; Vanderschuren *et al.* 2008).

MA is one of the most frequently abused drugs by pregnant women (Marwick 2000). In an IDEAL (Infant Development, Environment, and Lifestyle) study, 84.3% of 191 drug-abusing mothers admitted using MA in the first, 56% in the second, and 42.4% in the third trimester. In addition, 29.3% of mothers sustained a high frequency, 9.4% increased their frequency, 25.7% had a steady use frequency, and 35.6% had a decreased frequency of MA abuse during their pregnancy (Della Grotta *et al.* 2010). Therefore, information regarding the consequences of MA abuse during different periods of pregnancy may influence addicted mothers to stop using MA on behalf of their unborn child. The third goal of our study was to compare the effect of prenatal MA exposure during the first and second half of the prenatal development. During early embryogenesis, drugs can have teratogenic outcomes or lead to malformations (Acuff-Smith *et al.* 1996; Davidson *et al.* 2001). On the other hand, drugs can have more subtle consequences during the late fetal development period, when the main processes of organogenesis are complete, e.g., changes in brain formation, volume of neurotransmitters, expression of receptors, and atypical growth (Acuff-Smith *et al.* 1996; Rice and Barone 2000). As predicted, the present results have confirmed decreased social play elements, i.e., pinning and pouncing, in prenatal GD 1–11 rats compared with GD 12–22 rats, which contradicts our previous study, using the SIT, and the same drug exposure schedule with adults. Specifically, adult MA GD 12–22 rats spent less time in mutual sniffing, climbing, crawling, and following compared with the MA GD 1–11 rats (Hrebíčková *et al.* 2017).

Conclusion

In conclusion, the present study leads us to question whether the timing of prenatal MA exposure is crucial relative to changes in postnatal development, emotional, and executive processes. To the best of our knowledge, this is the first study examining the prenatal effect of a amphetamine-type stimulant on social play behavior and offers two primary outcomes. First, our results suggest that manipulation during the first half of prenatal development creates more

sensitivity to MA exposure and has a greater impact on social play behavior. Second, acute MA exposure can eliminate all social play behavior.

Acknowledgment

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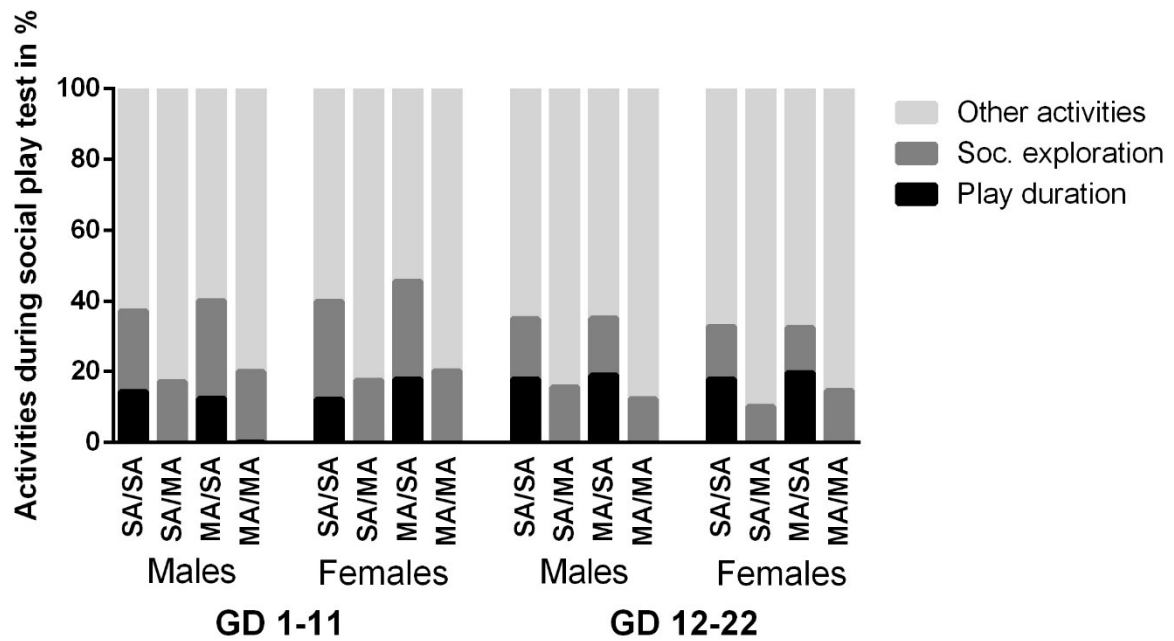
Figure 1 Proportion of activities during 15 min of social play test in percentage. Play duration includes pouncing, pinning and wrestling. Social exploration is the time of sniffing and grooming the partner or climbing over the partner. Other activities include space exploration (sniffing, rearing, and manipulating shavings), self-grooming or freezing. Groups are denoted as prenatal exposure/acute exposure. SA – saline exposure; MA – methamphetamine exposure; GD – gestational day.
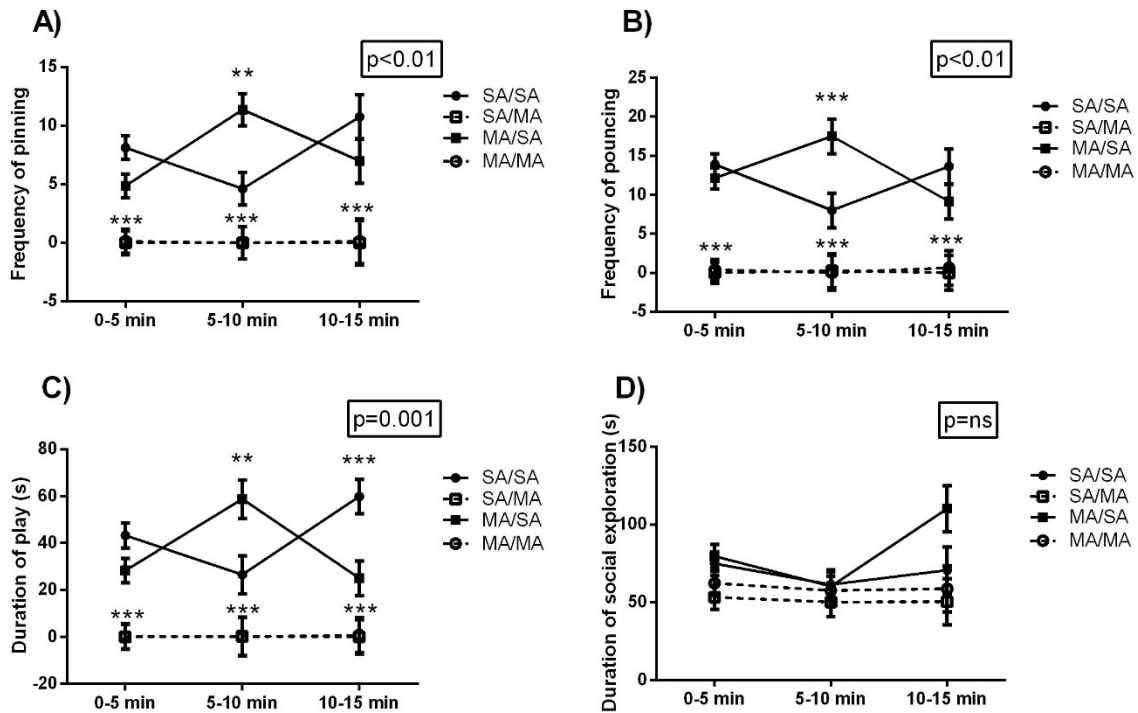
Figure 2 Effect of prenatal and acute MA exposure on the change of social play behavior in the group of males GD 1–11 over time, during three intervals (0-5 min, 5-10 min, 10-15 min). (A) The frequency of pinning, (B) The frequency of pouncing, (C) Total play duration, (D) Duration of social exploration. Values are means ± SEM (n = 8). The three-way ANOVA with repeated measures (*prenatal drug × acute drug × time*) was used for statistical analyze. The main effect is given in box, asterisks represents the differences with the SA/SA group revealed by post-hoc test. Groups are denoted as prenatal exposure/acute exposure. SA – saline exposure; MA – methamphetamine exposure; GD – gestational day.
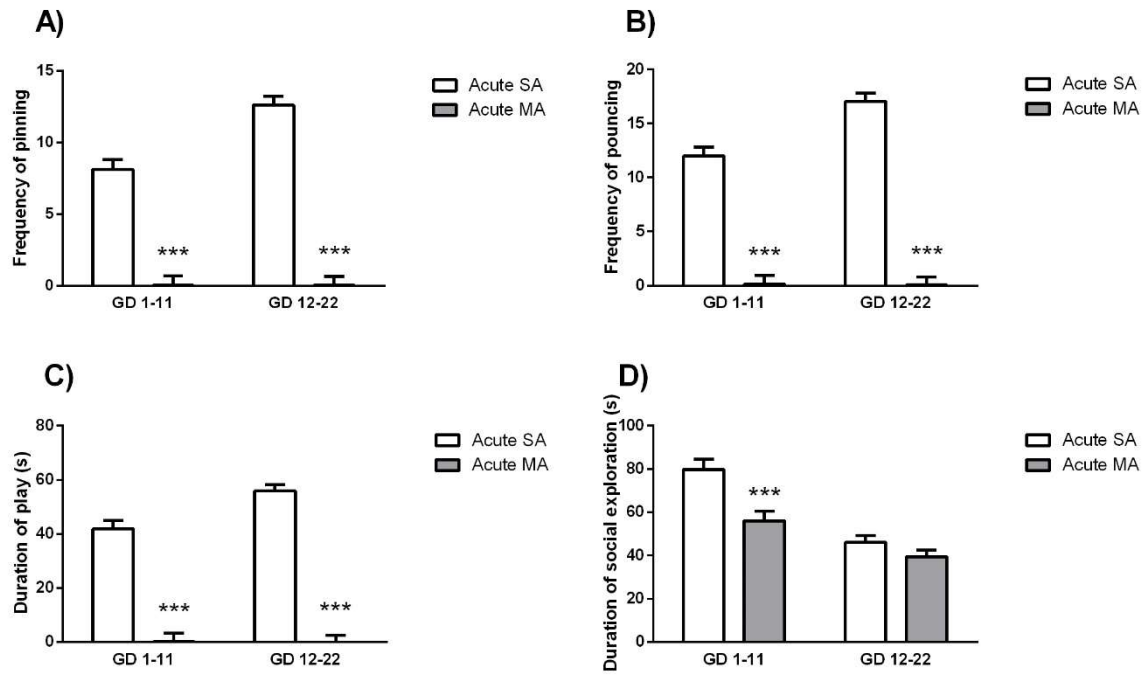
Figure 3 Effect of acute MA exposure on social play behavior. (A) The frequency of pinning, (B) The frequency of pouncing, (C) Total play duration, (D) Duration of social exploration. Values are means ± SEM (n = 32). Bars represent data regardless of sex and prenatal exposure period. The one-way ANOVA (*acute drug) was used for statistical analyze.* The main effect is given by asterisks: *** $p < 0.001$ acute MA vs. acute SA. Acute SA – acute saline exposure regardless of sex and prenatal drug exposure; Acute MA – acute methamphetamine exposure regardless of sex and prenatal drug exposure; GD – gestational day.
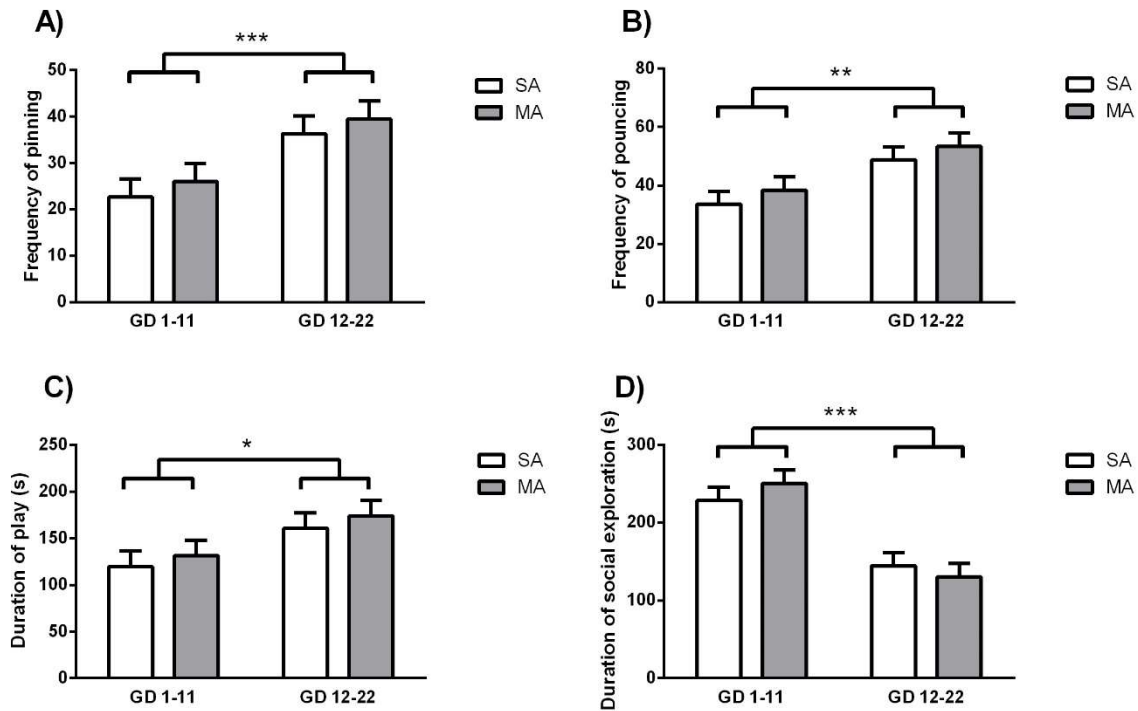
Figure 4 Effect of prenatal exposure period on social play behavior. (A) The frequency of pinning, (B) The frequency of pouncing, (C) Total play duration, (D) Duration of social exploration. Values are means ± SEM (n = 16). Bars represent data regardless of sex; groups with acute MA exposure were not used in the statistical evaluation. The one-way ANOVA *(exposure period)* was used for statistical analyze. The main effect is given by asterisks: * $p < 0.05$ exposure during GD 1-11 vs. GD 12-22 regardless the prenatal drug, ** $p < 0.01$ exposure during GD 1-11 vs. GD 12-22 regardless the drug, *** $p < 0.001$ exposure during GD 1-11 vs. GD 12-22 regardless the drug. SA – prenatal saline exposure; MA – prenatal methamphetamine exposure; GD – gestational day.