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The neurobiology of social play behaviour: Past, present and future

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ABSTRACT

Social play behaviour is a highly energetic and rewarding activity that is of great importance for the development of brain and behaviour. Social play is abundant during the juvenile and early adolescent phases of life, and it occurs in most mammalian species, as well as in certain birds and reptiles. To date, the majority of research into the neural mechanisms of social play behaviour has been performed in male rats. In the present review we summarize studies on the neurobiology of social play behaviour in rats, including work on pharmacological and genetic models for autism spectrum disorders, early life manipulations and environmental factors that influence play in rats. We describe several recent developments that expand the field, and highlight outstanding questions that may guide future studies.

1. Introduction

Social play behaviour has intrigued scientists for a long time, the first biological writings about it dating back to the late nineteenth century (Groos, 1898; Small, 1899). With the study of this energetic and highly rewarding activity, abundant in the young of most mammalian species, came the realization that social play is not just about having fun with others, but that it is also of great importance for the development of social, emotional and cognitive skills (Graham and Burghardt, 2010; Pellis and Pellis, 2009; Vanderschuren and Trezza, 2014). This made the study of the neural mechanisms of social play behaviour even more important. The first reviews about this topic (Panksepp et al., 1984; Thor and Holloway, 1984a) summarized the literature since the beginning of the systematic analysis of this behaviour in young male rats (Baenninger, 1967; Bolles and Woods, 1964; Meaney and Stewart, 1981; Panksepp and Beatty, 1980; Poole and Fish, 1975). In these reviews, the effects of social isolation, hormonal and pharmacological interventions and the first neural manipulations on social play were described. Whereas the pharmacological manipulations started to generate a picture about the involvement of neurotransmitters such as dopamine, serotonin, acetylcholine, adenosine, opioids and noradrenaline in social play, neurobiological manipulations consisted of either impairing sensory processes or electrolytic lesions of hypothalamic and limbic brain regions. In an updated review thirteen years later, the list of systemic pharmacological manipulations and lesion studies had grown considerably (Vanderschuren et al., 1997). The continued study of the neural underpinnings of social play in the twenty-first century lead to an increasingly detailed picture, that also included brain region-specific pharmacological manipulations (Manduca et al., 2021; Trezza et al., 2019; Vanderschuren et al., 2016). The purpose of the present review is to summarize studies on the neurobiology of social play behaviour in rats preceded by a brief introduction of the structure of rat social play. Furthermore, we provide an update of recent literature, including work on pharmacological and genetic models for autism spectrum disorders (ASD), early life manipulations and environmental factors influencing play behaviour in rats. We conclude by pointing out several recent developments that expand the field, and identify certain issues that deserve attention in future studies.

2. Social play behaviour in rats

In rats, an episode of social play behaviour starts when one animal solicits another rat to play, by attempting to nuzzle the nape of the neck of the other animal with its snout. This is called ‘pouncing’ or ‘nape contact’. The animal that is solicited can respond in different ways. For example, it can move away, which can result in the soliciting animal chasing the other. It can also turn around to face the other animal, which may lead to the animals pushing and wrestling. The solicited animal can also respond by rotating onto its back whereby the other animal comes to stand over it, which is termed ‘pinning’ (Panksepp and Beatty, 1980; Pellis and Pellis, 1998; Poole and Fish, 1975; Vanderschuren et al., 1997). Importantly, the rotation to the dorsal surface that results in

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pinning is in most cases an active act of the solicited animal, whereby this animal uses this position to gain access to the nape of the other animal. Gaining access to the nape of another animal is therefore thought to be the purpose of the playful act, and rolling onto its back is likely a means to prolong the playful interaction. Indeed, one of the most important characteristics of social play behaviour is its reciprocity, in the sense that there can be many reversals with regard to the on-top and on-bottom positions of the animals engaging in the interaction. Pinning and pouncing are considered to be the most characteristic expressions of social play behaviour in rats. They are easy to recognize and quantify and they are reliably stimulated by brief periods (i.e. hours) of social isolation (Hole, 1991; Panksepp and Beatty, 1980; Vanderschuren et al., 1995, 2008; Niesink and Van Ree, 1989).

Detailed analysis of the structure of social play behavior in rats by Pellis and colleagues (for reviews see Pellis et al., 1997, 2022, 2023; Pellis and Pellis, 1998) has shown that pinning is most abundant in juvenile rats (i.e., between weaning and the onset of puberty), which is also the age when social play in rats is most abundant (Panksepp, 1981). Hereafter, as the animals become sexually mature, a response to pouncing is more often followed by a partial rotation, whereby the animal maintains its hindlegs on the ground, which can lead to upright boxing or wrestling. This shift is more pronounced in male rats, indicating that the structure of social play is age- and sex-dependent.

For the study of the neural mechanisms of social play, the vast majority of studies described below have assessed play in a dyadic encounter, that is, the interaction between two animals. The experimental setup can differ in various ways (see 'A note on how social play is investigated: differences and similarities in methodology' below, and Pellis et al., 2022 for discussions), for example by testing familiar (i.e., cage- or littermates) or unfamiliar animals, experimentally treating both animals (in which case the test pair is one experimental unit), or one of them (whereby the other animal is the neutral stimulus partner, and only the behaviour of the treated animal is assessed), by varying the duration of social isolation before the test (in order to titrate towards a desired level of play behaviour during testing) and varying the test duration itself. In most cases in the following section, pinning and pouncing are used as the primary parameters of social play behaviour (sometimes only pinning is assessed, and sometimes various playful behaviours are summed together) and social exploratory behaviour (i.e., sniffing the body of the other animal) is used as a measure for non-playful social interest. Unless otherwise indicated, the reader can assume that pinning and pouncing (if both are scored) co-vary after pharmacological treatment, and that there are no treatment effects on social exploratory behaviour. The former is important, as changes in pinning but not pouncing (or vice versa) indicate that playful solicitation, or the responsiveness to play solicitation is altered as a result of treatment, rather than social play behaviour as a whole. The latter is important to distinguish treatment effects on social play behaviour from general changes in social behaviour. In addition, the lion's share of research on the neural mechanisms of social play behaviour in rats has been performed in male animals. Therefore, unless otherwise indicated, the studies below describe experiments in male rats.

3. The neurobiology of social play behaviour

3.1. Opioid neurotransmission

Perhaps the earliest pharmacological studies on the neural mechanisms of social play behaviour, published in the 1980 s, have used opioid drugs (Jalowiec et al., 1989; Niesink and Van Ree, 1989; Panksepp et al., 1980; Panksepp et al., 1985; Siegel and Jensen, 1986; Siegel et al., 1985). By and large, these studies demonstrated that treatment with an opioid receptor agonist such as morphine stimulates, and treatment with an opioid receptor antagonist such as naloxone inhibits social play behaviour in juvenile rats. These findings have been replicated several times in later years (Achterberg et al., 2019; Manduca et al., 2014; Manduca et al., 2016a; Román et al., 2021a; Trezza and Vanderschuren, 2008a; Trezza and Vanderschuren, 2008b; 2009; Vanderschuren et al., 1995a; Vanderschuren et al., 1995b). In this regard, the opioid system is the first neurotransmitter system that was implicated in the modulation of social play behaviour, which may not come as a surprise, given that social play is known to be a rewarding activity (Trezza et al., 2011a; Vanderschuren, 2010), and the well-known importance of opioid signaling for reward processes (Le Merrer et al., 2009; Van Ree et al., 1999).

The canonical view of the endogenous opioid system is that it comprises three classes of opioid molecules, i.e. endorphins, enkephalins and dynorphins, and three classes of receptors, termed mu, delta and kappa (Akil et al., 1984; Le Merrer et al., 2009). Of these, the mu opioid receptor appears to play the most prominent role in the modulation of social play. Morphine and naloxone are mu receptor-preferring drugs, as is the endogenous opioid beta-endorphin (Goldstein and Naidu, 1989), treatment with which (either systemically or into the nucleus accumbens) enhances social play behaviour (Niesink and Van Ree, 1989; Trezza et al., 2011b). Moreover, after systemic- or intra-accumbens treatment with a selective mu opioid receptor agonist social play increased, whereas treatment with a mu receptor antagonist reduced social play (Trezza et al., 2011b; Vanderschuren et al., 1995b). Conversely, systemic or intra-accumbens treatment with a kappa opioid receptor agonist suppressed social play behaviour (Trezza et al., 2011b; Vanderschuren et al., 1995b; Varlinskaya et al., 2018) as well as social investigation (Vanderschuren et al., 1995b; Varlinskaya et al., 2018), whereas treatment with delta opioid receptor drugs did not affect social play. In addition to the canonical opioids and its receptors, nociceptin/orphanin FQ is the endogenous agonist for the opioid-like nociceptin receptor (Meunier et al., 1995; Reinscheid et al., 1995), the anti-analgesic actions of which are typically thought of as opposite to endogenous opioids. Its involvement in social play behaviour remains to be studied, however.

As already alluded to above, the nucleus accumbens has been identified as an important site of action for stimulation of mu opioid receptors to enhance, and stimulation of kappa opioid receptors to reduce social play behaviour (Trezza et al., 2011b), supported by earlier work suggesting release of endogenous opioids in the nucleus accumbens during social play (Vanderschuren et al., 1995d). In this study (Trezza et al., 2011b), infusion of a mu-opioid receptor agonist into both the core and shell subregions of the nucleus accumbens stimulated social play, whereby lower doses of the drug were effective in the shell. Strikingly, Trezza et al. (2011b) demonstrated that intra-accumbens treatment with naloxone blocked the play-enhancing effect of systemic morphine treatment, indicating that stimulation of opioid receptors within the nucleus accumbens is necessary and sufficient for mu opioid receptor agonists to enhance social play behaviour. More recently, the medial preoptic area has also been implicated in the opioid modulation of social play behaviour in rats (Zhao et al., 2020). In this study, increased cellular activity of mu opioid receptor-expressing neurons in the medial preoptic area was observed after social play behaviour. Consistent with a physiological role of medial preoptic mu receptors in social play, knockdown of mu opioid receptors in this brain structure using short hairpin RNA reduced social play behaviour (Zhao et al., 2020).

With regard to the subcomponents of social play influenced by opioid treatment, the available data indicate that morphine treatment increases the initiation of social play (Trezza and Vanderschuren, 2008b) and solidifies its internal structure, thus prolonging bouts of social play (Vanderschuren et al., 1995c), but does not alter social play by increasing feelings of safety or reducing anxiety (Trezza and Vanderschuren, 2008b). These observations hint at the possibility that opioid neurotransmission modulates the positive emotions associated with social play, initial evidence for which was found in a study using a T-maze setup (Normansell and Panksepp, 1990). More recently, the role of opioid neurotransmission in social play reward and motivation was directly investigated using place conditioning and operant conditioning

setups, respectively (Achterberg et al., 2019). Here, treatment with naloxone reduced responding for social play under a progressive ratio schedule of reinforcement, and blocked the development of social play-induced conditioned place preference. The latter effect was previously also found after intra-nucleus accumbens treatment with a selective mu-opioid receptor antagonist (Trezza et al., 2011b). Conversely, treatment with morphine facilitated the induction of social play-induced place preference, but did not alter responding for social play, likely as a result of its rate-altering effects (Achterberg et al., 2019). Together, these data demonstrate that opioid neurotransmission is important for both the rewarding and motivational properties of social play behaviour.

Clearly, opioids do not affect social play in isolation, as interactions of opioids with other neuromodulator systems have been shown to influence social play. These have been well investigated in relation to the endocannabinoid system. Thus, the play-enhancing effects of indirect cannabinoid agonists could be prevented by pretreatment with naloxone. Vice versa, the play-enhancing effects of morphine were prevented by pretreatment with a cannabinoid receptor antagonist (Trezza and Vanderschuren, 2008a). Interestingly, pretreatment with morphine for several days blunted the increase in social play induced by treatment with a cannabinoid receptor agonist, but not vice versa (Schiavi et al., 2019). Later studies revealed the nucleus accumbens as a site of action for this opioid-cannabinoid interaction. That is, intra-accumbens pretreatment with a cannabinoid receptor antagonist prevented the stimulating effects of systemic morphine treatment on play, whereas intra-accumbens pretreatment with naloxone could prevent the positive effect of an indirect cannabinoid agonist on social play (Manduca et al., 2016a). Furthermore, nucleus accumbens dopamine has also been implicated in the play-enhancing effect of systemic morphine treatment, as this was blocked after intra-accumbens pretreatment with a dopamine receptor antagonist (Manduca et al., 2016b). Opioid receptor blockade with naloxone has also been shown to reduce the increase in social play induced by treatment with nicotine and alcohol (Trezza et al., 2009; Varlinskaya and Spear 2009).

In sum, there is a wealth of data to support an important role for opioid neurotransmission in the modulation of social play (see Table 1). Consistent with previous assumptions, opioids have recently been shown to be involved in the rewarding and motivational aspects of social play. With regard to the neural site of action, the nucleus accumbens is likely a key node for opioids to influence social play, whereas recent data implicate the medial preoptic area as well.

3.2. Cannabinoid neurotransmission

After the discovery of endogenous cannabinoids and their receptors in the late 1980s and early 1990s (Devane et al., 1988; Devane et al., 1992), investigation of the physiological role of endocannabinoid neurotransmission has taken enormous flight (for reviews see Lu and Mackie, 2016; Lutz et al., 2015; Mechoulam et al., 2014; Piomelli, 2003). Important for the present review is the reported involvement of cannabinoid signaling in reward processes (Parsons and Hurd, 2015; Solinas et al., 2008), and its interaction with other reward-related neuromodulator systems, including opioids and dopamine (Wenzel and Cheer, 2018). In brief, the endocannabinoid system comprises two major endocannabinoid molecules, i.e. anandamide and 2-arachidonoyl-glycerol (2-AG), their synthesizing enzymes, two receptors (CB1 and CB2), the membrane transporters that take them up into the cell after release and the enzymes that subsequently hydrolyze them – i.e. fatty acid amide hydrolase (FAAH) for anandamide and monoacylglycerol lipase (MAGL) for 2-AG, respectively (Mechoulam et al., 2014; Piomelli, 2003).

The first evidence to implicate cannabinoid signaling in the modulation of social play behaviour came from a study that showed bidirectional effects on play, depending on how cannabinoid neurotransmission was stimulated (Trezza and Vanderschuren, 2008a). That is, treatment with direct cannabinoid receptor agonists, such as Δ^9THC, WIN55,212–2 or methanandamide reduced social play (Trezza et al., 2014; Trezza and Vanderschuren, 2008a, 2009), whereas treatment with indirect agonists, i.e. drugs that inhibit the degradation of anandamide (i.e. the FAAH inhibitor URB597) or its reuptake (using VDM11), enhanced social play (Manduca et al., 2015b; Trezza et al., 2012; Trezza and Vanderschuren, 2008a; Trezza and Vanderschuren, 2008b, 2009). Importantly, both the play-suppressant effects of direct cannabinoid receptor agonists and the play-enhancing effects of cannabinoid receptor antagonists were prevented by pretreatment with the CB1 cannabinoid receptor antagonist SR141716A. Intriguingly, treatment with WIN55,212–2 was found to reduce social play behaviour even 24 h post-treatment, but in female rats only (Borsoi et al., 2019). Moreover, repeated treatment with Δ^9THC reduced social play in both sexes (Keeley et al., 2021). Together, these data indicated that widespread activation of CB1 cannabinoid receptors throughout the brain causes effects on emotion and cognition that inhibit the appropriate execution of playful behaviours. On the other hand, the play-stimulating effects of indirect cannabinoid agonists suggest that during social play, anandamide release in brain structures relevant for social play facilitates this behaviour. Therefore, prolonging the availability of anandamide in the extracellular space enhances social play.

More recently, the other main endocannabinoid molecule 2-AG has also been implicated in social play behaviour. Treatment with JZL195, a drug that inhibits both FAAH and MAGL, the enzymes that degrade anandamide and 2-AG, respectively, was shown to increase social play (Manduca et al., 2015a). Importantly, the effect on play was associated with an increase in brain levels of 2-AG, but not anandamide. Follow-up studies demonstrated that treatment with JZL184, which selectively inhibits MAGL, also increases social play (Manduca et al., 2016a; Schiavi et al., 2019), providing direct evidence for the involvement of 2-AG. In somewhat older rats, the MAGL inhibitor MJN110 did not alter social play in either sex (Fontenot et al., 2018), suggesting that the role of 2-AG in play may be age-dependent, although the observation that JZL195-treatment increased playful – as well as non-playful – social behaviour in adult rats (Manduca et al., 2015a) argues against this possibility.

Cannabinoids have been shown to influence social play behaviour through their actions in corticolimbic brain structures. Social play evoked increases in anandamide levels in the nucleus accumbens, dorsal striatum and amygdala, but not prefrontal cortex or hippocampus (Marco et al., 2011; Trezza et al., 2012). Importantly, URB597 treatment increased anandamide levels further and social play also increased phosphorylation of CB1 receptors (a proxy for CB1 receptor activation) in the amygdala, but not the accumbens. Treatment with URB597 into the nucleus accumbens and the basolateral amygdala increased social play. Furthermore, treatment with the CB1 cannabinoid receptor antagonist SR141716A into the basolateral amygdala prevented the play-enhancing effect of systemic URB597 treatment, but this effect was not observed after intra-accumbens SR141716A-treatment (Trezza et al., 2012). These data implicate the basolateral amygdala, and to a lesser extent the nucleus accumbens and dorsal striatum in the positive influence of anandamide release on social play behaviour. The nucleus accumbens also appears to be important for the play-enhancing properties of 2-AG release. Thus, intra-accumbens pretreatment with SR141716A prevented the play-enhancing effect of systemic JZL184 treatment (Manduca et al., 2016a). Moreover, after treatment with JZL184, that increased social play, an increase in phosphorylation of the CB1 receptor and its effector protein Akt was found in the nucleus accumbens and prefrontal cortex (Schiavi et al., 2019). Together, these findings provide evidence for an involvement of the nucleus accumbens and basolateral amygdala in the facilitating effects of endocannabinoids on social play behaviour, whereby the former appears to be the neural site of action of 2-AG, and the latter of anandamide, respectively, although there is probably not a strict distinction. Furthermore, the prefrontal cortex and dorsal striatum may also be neural substrates of

Table 1
Effect of acute treatment with drugs affecting opioid, cannabinoid, dopamine, noradrenaline, serotonin and vasopressin neurotransmission on social play behaviour.

Drug	Action[a]	Target[b]	Strain[c]	Age play tested (PND)[d]	Isolation procedure (hrs)[e]	Play observation time (min)	Type of partner[f]	Effect on play[g]	Dose	Reference
Opioids										
Morphine	μ ag	s	w, le, sd	21,23,26,28,30,35–37	0, 1, 3.5, 24, PND21-intire exp	10 × 1, 5–15	wm, u/f, sm, st	↑	1–3 mg/kg	Niesink and Van Ree (1989);Normansell and Panksepp (1990);Panksepp et al. (1980);Panksepp et al. (1985);Vanderschuren et al. (1995a); c; Trezza and Vanderschuren, -b (2008a);Trezza et al. (2011b);Manduca et al., (2014, 2016b); Achterberg et al. (2019);Schiavi et al. (2019); Román et al., 2021
		nac	w	37 and 39	2	15	wm, u, sm, st	↑	0.05–0.1 μg/ 0.3 μl	Trezza et al. (2011b),Manduca et al. (2016b)
β-endorphin	μ ag	s	w	28	0, 1.75, 3.5, 24	15	wm, u, sm, st	↑/↓	10 μg/100 μg	Niesink and Van Ree (1989)
		nac	w	37 and 39	2	15	wm, u, sm, st	↑	0.01–1 μg/ 0.3 μl	Trezza et al. (2011b)
Methadone	μ ag	s	w	21	3.5	15	wm, u, sm, st	↑	0.3 mg/kg	Vanderschuren et al. (1997)
Fentanyl	μ ag	s	w	21	3.5	15	wm, u, sm, st	↑	0.01–0.03 mg/kg	Vanderschuren et al. (1995a)
DAMGO	μ ag	nac	w	37 and 39	2	15	wm, u, sm, st	↑	0.1–10 ng/ 0.3 μl	Trezza et al. (2011b)
Naloxone	μ ant	s	w, le	21, 33–37	cont	0, 10 × 1, 5	wm, u, sm, st	↓	0.5–10 mg/kg	Beatty and Castello, 1982;Normansell and Panksepp (1990);Panksepp et al. (1980); Panksepp et al., 1981; Siegel et al. (1985); Siegel et al., 1986; Siviy and Panksepp, 1985;Trezza and Vanderschuren (2009);Achterberg et al. (2019)
		nac	w	37 and 39	2	15	wm, u, sm, st	↓	0.5 μg/ 0.3 μl	Trezza et al. (2011b);Manduca et al. (2016b)
Naltrexone	μ ant	s	w	28	3.5	15	wm, u, sm, st	↓	0.1–1 mg/kg	Jalowiec et al. (1989);Niesink and Van Ree (1989)
CTAP	μ ant	nac	w	37 and 39	2	15	wm, u, sm, st	↓	0.3–3 μg/ 0.3 μl	Trezza et al. (2011b);Manduca et al. (2016b)
met-enkephalin	μ ant	nac	w	37 and 39	2	15	wm, u, sm, st	-	0.1–5 μg/0.3 μl	Trezza et al. (2011b)
β-Funaltrexamine	μ ant	s	w	21	3.5	15	wm, u, sm, st	↓	3.0 mg/kg	Vanderschuren et al. (1995a)
Knock-down	μ s	mpoa	sd	36–37	24	20	4 males, 1 female	↓	1.7 × 10^13 GC/mL, 0.5 μl	Zhao et al. (2020)
U69593	κ ag	nac	w	37 and 39	2	15	wm, u, sm, st	↓	0.01–1 μg/ 0.3 μl	Trezza et al. (2011b)
U50,488 H	κ ag	s	w	21	3.5	15	wm, u, sm, st	↓	1.0–3.0 mg/ kg	Vanderschuren et al. (1995a)
Nor-binaltorphimine	κ ant	s	w	21	3.5	15	wm, u, sm, st	-	0.1–3.0 mg/ kg	Vanderschuren et al. (1995a)
BUBUC	δ ag	s	w	21	3.5	15	wm, u, sm, st	-	0.1–1.0 mg/ kg	Vanderschuren et al. (1995a)
Naltrindole	δ ant	s	w	21	3.5	15	wm, u, sm, st	-	0.3–3.0 mg/ kg	Vanderschuren et al. (1995a)
DPDPE	δ ag	nac	w	37 and 39	2	15	wm, u, sm, st	-	0.3–3 μg/ 0.3 μl	Trezza et al. (2011b)
Cannabinoids										
Δ9THC	direct ag	s	w	28	3.5	15	wm, u, sm, st	↓	0.5–5 mg/kg	Trezza et al. (2014);Keeley et al. (2021)

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Table 1 (*continued*)

Drug	Action[a]	Target[b]	Strain[c]	Age play tested (PND)[d]	Isolation procedure (hrs)[e]	Play observation time (min)	Type of partner[f]	Effect on play[g]	Dose	Reference
WIN55,212–2	direct ag	s	w	28, 34–37, 47–51	3.5, 24	15	wm, u, sm, st	-males/ ↓females	0.3–2 mg/kg	Borsoi et al. (2019);Trezza and Vanderschuren (2008a); b
(R)-methanandamide	direct ag	s	w	28	3.5	15	wm, u, sm, st	↓	0.3–3 mg/kg	Trezza and Vanderschuren (2009)
URB597	indirect ags, FAAH inh	s	w	35–37	3.5	15	wm, u, sm, st	↑	0.05–0.2 mg/kg	Manduca et al. (2015b), (2016b);Trezza et al. (2012);Trezza and Vanderschuren (2008a); b; 2009
		s	w	32–45	2, 24	5–15 in bouts of 1	wm, u, sm, st	↓ motivation	0.05–0.2 mg/kg	Achterberg et al., 2016
		nac	w	30–32	2	15	wm, u, sm, st	↑	0.005–0.01 μg/ 0.3 μl	Trezza et al. (2012)
		amy	w	30–32	2	15	wm, u, sm, st	↑	0.005–0.01 μg/ 0.3 μl	Trezza et al. (2012)
AM404	reuptake inh	s	w	28	3.5	15	wm, u, sm, st	↓	0.5–5 mg/kg	Trezza and Vanderschuren (2009)
JZL184	MAGL inh	s	w	35–37	3.5	15	wm, u, sm, st	↑	1–16 mg/kg	Manduca et al. (2016a);Schiavi et al. (2019)
MJN110	MAGL inh	s	sd	50	2	15	wm, u, sm	-	1–5 mg/kg	Fontenot et al. (2018)
JZL195	FAAH and MAGL inh	s	sd	28–35–37	3.5, 24	15	wm, u, sm, st	↑	0.01–1 mg/kg	Manduca et al. (2015a)
VDM11	indirect ag/ reuptake inh	s	w	28	3.5	15	wm, u, sm, st, yp	↑	0.5–1 mg/kg	Trezza and Vanderschuren (2009)
SR141716A	CB1 ant	s	w	28, 35–37	3.5	15	wm, u, sm, st	↓	0.1–1 mg/kg	Trezza and Vanderschuren (2009);Manduca et al. (2016a)
		s	w	32–45	24	5–15 in bouts of 1	wm, u, sm, st	↓ motivation	0.05–0.2 mg/kg	Achterberg et al., 2016
		nac	w	30–32,35–37	2	15	wm, u, sm, st	-	0.3–3 μg/0.3 μl	Trezza et al. (2012);Manduca et al. (2016b)
		amy	w	35–37	2	15	wm, u, sm, st	↓	0.03–3 μg/ 0.2 μl	Trezza et al. (2012)
SR144528	CB2 ant	s	w	28	3.5	15	wm, u, sm, st	-	0.1 mg/kg	Trezza and Vanderschuren (2009)
Psychostimulants (DA/NA/5HT)										
Amphetamine	DA/NA rel and reuptake inh	s	w, sd, alb, le	26–42	3.5, 24, 6 days, continuouos isolation	10, 60	wm, u, sm, st/ nis	↓	0.05–1.0 mg/kg	Beatty et al., (1982, 1984);Thor and Holloway (1983);Sutton and Raskin (1986);Achterberg et al. (2014)
		nac	w	35–37	2	15	wm, u, sm, st	↑	0.03–1 μg/ 0.3 μl	Manduca et al. (2016b)
Methylphenidate	DA/NA reuptake inh	s	w, alb, le	26–42	3.5, 24, 6 days	10, 15	wm, u, sm, st/ nis	↓	0.5–4 mg/kg	Beatty et al., (1982, 1984);Thor and Holloway (1983);Vanderschuren et al. (2008);Achterberg et al., (2015, 2016a)
		s	w	29–45	24	5, 45	wm, u, sm, st	↑ motivation	1–3 mg/kg	Achterberg et al. (2016a)
		acc	w	29–34	2.5	15	wm, u, sm, st	↓	5 μg/0.3 μl	Achterberg et al. (2015)
		prl	w	29–34	2.5	15	wm, u, sm, st	-	5 μg/0.3 μl	Achterberg et al. (2015)
		il	w	29–34	2.5	15	wm, u, sm, st	↓	5 μg/0.3 μl	Achterberg et al. (2015)

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Table 1 (*continued*)

Drug	Action[a]	Target[b]	Strain[c]	Age play tested (PND)[d]	Isolation procedure (hrs)[e]	Play observation time (min)	Type of partner[f]	Effect on play[g]	Dose	Reference
		mo/vo	w	29–34	2.5	15	wm, u, sm, st	-	5 μg/0.3 μl	Achterberg et al. (2015)
		vlo	w	29–34	2.5	15	wm, u, sm, st	-	5 μg/0.3 μl	Achterberg et al. (2015)
		nacs	w	29–34	2.5	15	wm, u, sm, st	-	5 μg/0.3 μl	Achterberg et al. (2015)
		bla	w	29–34	2.5	15	wm, u, sm, st	↓	5 μg/0.3 μl	Achterberg et al. (2015)
		hab	w	29–34	2.5	15	wm, u, sm, st	↓	5 μg/0.3 μl	Achterberg et al. (2015)
		mdt	w	29–34	2.5	15	wm, u, sm, st	-	5 μg/0.3 μl	Achterberg et al. (2015)
Cocaine	DA/NA reuptake inh	s	w, sd	29–35	0.5, 3.5	5, 15	wm, u/f, sm, st	↓	0–20 mg/kg	Ferguson et al. (2000);Thiel et al. (2008); Achterberg et al., (2014, 2016a)
		s	w	29–45	24	5, 45	wm, u, sm, st	↑ motivation	5–10 mg/kg	Achterberg et al. (2016a)
Methylenedioxypyrovalerone	DA/NA reuptake inh	s	w	28–30	3.5	15	wm, u, sm, st/vt	↓	0.025–0.5 mg/kg	Schiavi et al. (2020)
Dopamine (DA)										
GBR-12909	Selective DA reuptake inh	s	w	35–37	24	15	wm, u, sm, st	-	0–10 mg/kg	Vanderschuren et al. (2008); Roman et al., 2021
		s	w	29–45	24	5, 45	wm, u, sm, st	↑ motivation	3–10 mg/kg	Achterberg et al. (2016a)
		nac	w	35–37	2	15	wm, u, sm, st	-	0.1–3 μg/0.3 μl	Manduca et al. (2016b)
Apomorphine	DA ag	s	w	28	3	15	wm, u, sm, st	↓	10–100 μg/kg	Niesink and Van Ree (1989)
		s	alb	26–33	24	10	wm, u, sm, st	↑	0.03–1 mg/kg	Beatty et al. (1984);Vanderschuren et al. (2008)
		nac	w	35–37	2	15	wm, u, sm, st	↑	0.01–0.1 μg/0.3 μl	Manduca et al. (2016b)
α/cis-z-flupenthixol	DA ant	s	w	28	3.5	15	wm, u, sm, st	↓	0.125–0.5 mg/kg	Vanderschuren et al. (2008);Trezza and Vanderschuren (2009);Achterberg et al., (2014, 2016a);Schiavi et al. (2020)
		nac	w	35–37	2	15	wm, u, sm, st	↓	7.5–30 μg/0.3 μl	Manduca et al. (2016b)
		ls	w	32	cont	10	am, sm, sh, u	↓	15–30 μg/0.5 μl	Bredewold et al. (2018)
Chlorpromazine	DA ant	s	alb	26–33	24	10	wm, u, sm, st	↓	0.5 – 5.0 mg/kg	Beatty et al. (1984); Einon et al., 1978;Humphreys and Einon (1981)
α-Methyltherosine	DA synthesis inh	s	alb	26–33	24	10	wm, u, sm, st	↓	50 mg/kg	Beatty et al. (1984)
SKF 38393	D1-DA ag	s	sd	25–45	cont	5	sm, st, f	↓	10 mg/kg	Siviy et al. (1996)
SCH 23390	D1-DA ant	s	sd	25–45	cont	5	sm, st, f	-	0.025 mg/kg	Siviy et al. (1996)
		s	sd	25–45	cont	5	sm, st, f	↓	0.1 mg/kg	Siviy et al. (1996)
		nac	w	35–37	2	15	wm, u, sm, st	↓	0.1–0.6 μg/0.3 μl	Manduca et al. (2016b)
Quinelorane	D2-DA ag	s	sd	25–45	cont	5	sm, st, f	↑	0.003 mg/kg	Siviy et al. (1996)

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Table 1 (*continued*)

Drug	Action[a]	Target[b]	Strain[c]	Age play tested (PND)[d]	Isolation procedure (hrs)[e]	Play observation time (min)	Type of partner[f]	Effect on play[g]	Dose	Reference
		s	sd	25–45	cont	5	sm, st, f	↓	0.03–0.1 mg/kg	Siviy et al. (1996)
Haloperidol	D2-DA ant	s	w, alb	26–33	3, 24	10, 15	wm, u, sm, st	↓	5 and 25 μg/kg	Beatty et al. (1984);Niesink and Van Ree (1989)
Quinpirole	D2-DA ag	s	sd	25–45	cont	5	sm, st, f	-	0–50 μg/kg	Siviy et al. (1996)
		s	sd	25–45	cont	5	sm, st, f	↓	100–200 μg/kg	Siviy et al. (1996)
Eticlopride	D2-DA ant	s	sd	25–45	cont	5	sm, st, f	↓	0.05–0.4 mg/kg	Siviy et al. (1996)
		nac	w	35–37	2	15	wm, u, sm, st	↓	0.1–15 μg/0.3 μl	Manduca et al. (2016b)
Haloperidol	D2-DA ant	s	alb	26–33	24	10	wm, u, sm, st	↓	0.025–10 mg/kg	Beatty et al. (1984);Holloway and Thor (1985); Niesink and Van Ree (1989)
Noradrenaline (NA)										
Noradrenaline	NA ag	ls	w	32	cont	10	am, sm sh, u	-	10–30 nmol/0.5 μl	Bredewold et al. (2018)
Atomoxetine	NA reuptake inh	s	w	35–37	3.5, 24	15	wm, u, sm, st	↓	0.3–3 mg/kg	Vanderschuren et al. (2008); Roman et al., 2021
		s	w	29–45	24	5, 45	wm, u, sm, st	↓ motivation	0.3–3 mg/kg	Achterberg et al. (2016a)
		acc	w	29–34	2.5	15	wm, u, sm, st	↓	10 μg/0.3 μl	Achterberg et al. (2015)
		il	w	29–34	2.5	15	wm, u, sm, st	↓	10 μg/0.3 μl	Achterberg et al. (2015)
		bla	w	29–34	2.5	15	wm, u, sm, st	↓	10 μg/0.3 μl	Achterberg et al. (2015)
		hab	w	29–34	2.5	15	wm, u, sm, st	↓	10 μg/0.3 μl	Achterberg et al. (2015)
Ephedrine	α-β-NA ag	s	alb	26–33	24	10	wm, u, sm, st	↑	10–80 mg/kg	Beatty et al. (1984);
Phentolamine	α-NA ant	ls	w	32	cont	10	am, sm sh, u	-	1–5 μg/0.5 μl	Bredewold et al. (2018)
Phenoxybenzamine	α-NA ant	s	alb	26–33	24	10	wm, u, sm, st	↓	10–20 mg/kg	Beatty et al. (1984);
St 587	α1-NA ag	s	sd	25–45	cont	5	sm, st, f	-	0.5–1.0 mg/kg	Siviy et al. (1994)
Clonidine	α2-NA ag	s	alb, le, sd	22–33	24, cont	5, 10	wm, u, sm, st	↓	0.0005–0.2 mg/kg	Beatty et al. (1984); Normansell and Panksepp, 1985;Siviy and Baliko (2000)
Prazosin	α1-NA ant	s	sd	25–45	cont	5	sm, st, f	↓	0.1–1.0 mg/kg	Siviy et al. (1994);Vanderschuren et al. (2008)
Yohimbine	α2-NA ant	s	le	25–45	cont	5	sm, st, f	-	0.3–5.0 mg/kg	Normansell and Panksepp, 1985
Idazoxan	α2-NA ant	s	sd	25–50	cont	5	sm, st, f	↑	1.0–8.0 mg/kg	Siviy et al. (1990);Siviy et al. (1994)
RX821002	α2-NA ant	s	w, sd	22–45	2.5, 3.5, 4, cont	jan-00	sm, st, f	↑	0.05–0.4 mg/kg	Siviy et al. (1994);Siviy and Baliko (2000); Vanderschuren et al. (2008);Achterberg et al., (2014, 2015, 2016a);Schiavi et al. (2020)
		acc	w	29–34	2.5	15	wm, u, sm, st	-	0.1 ng/0.3 μl	Achterberg et al. (2018)
		il	w	29–34	2.5	15	wm, u, sm, st	-	0.1 ng/0.3 μl	Achterberg et al. (2018)
		bla	w	29–34	2.5	15	wm, u, sm, st	-	0.1 ng/0.3 μl	Achterberg et al. (2018)

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Table 1 (*continued*)

Drug	Action[a]	Target[b]	Strain[c]	Age play tested (PND)[d]	Isolation procedure (hrs)[e]	Play observation time (min)	Type of partner[f]	Effect on play[g]	Dose	Reference
		hab	w	29–34	2.5	15	wm, u, sm, st	-	0.1–0.2 ng/ 0.3 µl	Achterberg et al. (2018)
Propranolol	β-NA ant	s	alb, w	26–33	3.5, 24	5, 10	wm, u, sm, st	↓	3.0–20 mg/kg	Beatty et al. (1984);Vanderschuren et al. (2008)
		ls	w	32	cont	10	am, sm, sh, u	-	2–5 µg/0.5 µl	Bredewold et al. (2018)
Serotonin (5HT)										
MDMA	5HT reuptake inh	s	w	28–35–37	3.5, 24	15	wm, u, sm, st	↓	0.5–5 mg/kg	Homberg et al. (2007); Roman et al., 2021
Fluoxetine	5HT reuptake inh	s	w, le	28–35–37	3.5, cont	5, 15	wm, u, sm, st	↓	1–10 mg/kg	Homberg et al. (2007);Knutson et al. (1996); Achterberg et al. (2014)
8-OH-DPAT	5HT1a ag	s	sd	22	4, 24	5	wm, f, sm, st	↓	0.01–0.3 mg/ kg	Siviy et al. (2011)
Fluprazine	5HT 1b/2c ag	s	sd	35–38	cont	10	wm, f, sm, st	↑/↓	4.0/ 8.0 mg/ kg	Panksepp, 1993, Selseth and Kemble 1988
Quipazine	5HT2 ag	s	le	23–28	cont	5	wm, f, sm, st	↓	1.0–10 mg/kg	Normansell and Panksepp, 1985
WAY100365	5HT1a ant	s	w	29–34	3.5	15	wm, u, sm, st	-	0.1 mg/kg	Achterberg et al. (2014)
Methysergide	5HT1b/1d/ 2 ant	s	w, le	22–34	3.5, cont	5, 15	wm, u/f, sm, st	↓	5.0–10 mg/kg	Normansell and Panksepp (1985b);Achterberg et al. (2014)
M100907	5HT2a ant	s	w	29–34	3.5	15	wm, u, sm, st	-	0.2 mg/kg	Achterberg et al. (2014)
Amperozide	5HT2 ant	s	w	29–34	3.5	15	wm, u, sm, st	-	0.5 mg/kg	Achterberg et al. (2014)
Ondansetron	5HT3 ant	s	w	29–34	3.5	15	wm, u, sm, st	-	1 mg/kg	Achterberg et al. (2014)
Vasopressin (AVP)										
synthetic AVP	AVP ag	icv	w	35–42	cont	10	wm, am, sm, u, ut	-	200 pg/0.5 µl	Veenema et al. (2013)
		ls	w	33–35	cont	10	wm, am, sm, u, ut	↓ females in novel environment	200 pg/0.5 µl	Bredewold et al. (2014)
(d(CH2)5-[Tyr(Me)2]AVP	V1a ant	icv	w	35–42	cont	10	wm, am, sm, u, ut	↓ males ↑ females	0.75 µg/5 µl	Veenema et al. (2013)
		ls	w	33–35	cont	10	wm, am, sm, u, ut	↑ males ↓ females	10 ng/0.5 µl	Bredewold et al., (2014, 2018)

[a] Ag: receptor agonist, ant: receptor antagonist, inh: inhibitor, rel: releaser

[b] S: systemic treatment, nac: nucleus accumbens, nacs: nucleus accumbens shell, acc: anterior cingulate cortex, prl: prelimbic cortex, il: infralimbic cortex, mo/vo: medial/ventral orbitofrontal cortex, vlo: ventrolateral orbitofrontal cortex, bla: basolateral amygdala, hab: habenula, mdt: mediodorsal thalamus, ls: lateral septum, mpoa: medial preoptic area, icv: intra-cerebroventricular

[c] W: Wistar, sd: Sprague-Dawley, alb: albino, le: Long-Evans, F344: Fischer 344

[d] PND: Post-natal day

[e] Hrs: hours, cont: continuously

[f] am: age matched, f: familiar, lm: littermate, nis: non-isolated stimulus animal, sg: similar genotype, sh: socially housed, sm: sex matched, ss: similar strain, st: similar treatment, u: unfamiliar ut: untreated, vt: vehicle treatment, wm: weight matched, yp: younger partner

[g] ↑: increase, ↓: decrease, - no effect

cannabinoid modulation of social play behaviour.

Being a rewarding activity, and cannabinoids having been implicated in reward processes, it is plausible to think that endocannabinoids enhance social play behaviour by boosting its positive emotional properties. When this assumption was formally tested however, support for it was not found. That is, treatment with neither the FAAH inhibitor URB597 nor the CB1 cannabinoid receptor antagonist SR141716A altered the induction of social play-induced conditioned place preference or had a profound effect on operant responding for social play (Achterberg et al., 2016b). At relatively high doses, URB597 and SR141716A both reduced responding for play, but this likely due to rate-altering effects, or behavioural competition, since SR141716A evoked vigorous scratching during the test. Previous work has also excluded the possibility that cannabinoid receptor agonists such as URB597 indirectly increase social play behaviour as a result of anxiolytic effects, since this drug was equally effective in both familiar and unfamiliar environments, and low and bright light circumstances (Manduca et al., 2015b; Trezza and Vanderschuren, 2008b). Indeed, at the dose that increased social play behaviour, the FAAH/MAGL inhibitor JZL195 did not alter behaviour on the elevated plus maze, although it did evoke anxiolytic effects at higher doses – that did not influence play (Manduca et al., 2015a). In addition, the delayed play-suppressant effects of the cannabinoid receptor agonist WIN55,212–2 were not associated with changes in locomotion or anxiety (Borsoi et al., 2019). Analysis of the structure of social play revealed that treatment with URB597 increased play solicitation, and also the responsiveness to play solicitation, although the latter effect required an equally motivated (i.e. URB597-treated) partner (Trezza and Vanderschuren, 2008b). In sum, the social play-enhancing properties of cannabinoid agonists are likely the result of an effect on the internal structure of social play, thus prolonging the playful interaction without specifically modulating one particular subcomponent of social play, although the effects of URB597 on play solicitation do indicate an influence on social initiative.

There is quite a bit of interest in the developmental effects of cannabinoid exposure on social play behaviour. By and large, studies on this topic show that the way cannabinoid treatment alters later social play is strongly dependent on the developmental period in which this takes place. The earliest of these studies showed that perinatal (from gestational day (GD) 15 until postnatal day (PND) 9) treatment with Δ^9THC reduced juvenile social play (Trezza et al., 2008). However, prenatal (GD5–20) treatment with the cannabinoid receptor agonist WIN55,212–2 did not affect social play (Manduca et al., 2020). Neonatal exposure to cannabinoids then, has been implicated in the sex-dependent development of social play, since neonatal (PND0–3) exposure to WIN55,212–2 increased later play in females, whereas treatment with a mixture of the CB1 cannabinoid receptor antagonist AM281 and the CB2 cannabinoid receptor antagonist AM630 reduced later play in males (Argue et al., 2017). These changes in play were associated with morphological alterations in the medial amygdala, resonating with early work implicating the amygdala in the sex-specific development of social play behaviour (Meaney et al., 1981). Interestingly, in the latter study (Argue et al., 2017) there were also modest reductions in play initiation in male rats after neonatal cannabinoid receptor agonist treatment, and increases in male rats after antagonist treatment, suggesting that the cannabinoid-driven sex-specific development of play is not quite straightforward. Underscoring the time-dependence of the impact of early cannabinoid exposure on play behaviour are two studies demonstrating that later postnatal cannabinoid agonist treatment actually enhances juvenile social play (Carr et al., 2020; Mohammed et al., 2018). The first one of these demonstrated that treatment of male and female rats with Δ^9THC from PND10–16 – i.e. immediately before the developmental onset of social play – increased social play during the transition from juvenile to adolescent age (PND37–38) (Mohammed et al., 2018). These effects were associated with reduced anxiety (but only in males) and hyperactivity. In the other study (Carr et al., 2020), rats were treated with the organophosphorus insecticide chlorpyrifos, which inhibits FAAH, as well as with the FAAH inhibitor PF-04457845, also from PND10–16. Treatment with both compounds increased social play on PND35 in males and females, reduced anxiety and increased locomotor activity.

The influence of cannabinoid agonists likely occurs in interaction with other neurotransmitter systems, most prominently opioids and dopamine. The latter is not surprising, given that these three neuromodulator systems have all been implicated in reward processes, and that they have been shown to interact in this regard (Fattore et al., 2010; Wenzel and Cheer, 2018). Previous studies have shown that the enhancements in social play after treatment with indirect cannabinoid agonists can be attenuated by pretreatment with dopamine or opioid receptor antagonists (Trezza and Vanderschuren, 2008a, 2009), whereby the dopamine-dependent aspect of cannabinoid-mediated increases in play likely occurs within the nucleus accumbens (Manduca et al., 2016b). More recently, the nucleus accumbens was also identified as a site of action for opioid-cannabinoid interactions in the modulation of social play. Thus, pretreatment with the opioid receptor antagonist naloxone or the selective mu-opioid receptor antagonist CTAP, was shown to attenuate the play-enhancing effect of systemic treatment with the MAGL inhibitor JZL184 (Manduca et al., 2016a). Underscoring the importance of opioid-cannabinoid interactions, Schiavi et al. (2019) demonstrated that the increase in play after treatment with JZL184 was blunted after morphine pretreatment – but not vice versa – suggesting unidirectional cross-tolerance between the effects of cannabinoid and opioid agonists on play. Last, cannabinoid neurotransmission is involved in the play-facilitating effects of morphine, nicotine and alcohol, as these could be prevented by treatment with a cannabinoid receptor antagonist (Trezza et al., 2009a; Trezza and Vanderschuren, 2008a, 2009).

In sum, cannabinoid neurotransmission has been widely implicated in the modulation of social play behaviour (see Table 1), whereby the importance of local endocannabinoid neurotransmission needs to be emphasized, as only indirect (but not direct) cannabinoid agonist treatment increases social play behaviour. This occurs in close interaction with opioid and dopamine neurotransmission. Cannabinoids have been shown to exert their positive effects on social play through the nucleus accumbens and basolateral amygdala, and to a lesser extent the prefrontal cortex and dorsal striatum, and these effects are not the by-product of changes in anxiety or locomotor activity. However, to date, direct evidence that cannabinoid neurotransmission modulates the positive emotional – motivational or pleasurable – properties of play is lacking. Last, early (pre-, peri- or postnatal) exposure to cannabinoid drugs has been shown to alter juvenile social play, whereby the direction of the effects depends on the way in which the cannabinoid system was stimulated, as well as the developmental phase in which this occurs.

3.3. *Dopamine neurotransmission*

Comprising ascending projections from two mesencephalic structures, i.e. the substantia nigra pars compact and the ventral tegmental area to forebrain regions including the dorsal striatum, nucleus accumbens, prefrontal cortex and amygdala (Björklund and Dunnett, 2007; Dahlström and Fuxe, 1964; Moore and Bloom, 1978; Ungerstedt, 1971), dopaminergic neurotransmission has been implicated in a variety of processes that may contribute to social play behaviour. That is, dopamine in the nucleus accumbens is thought to be involved in motivation, incentive salience, reward prediction error signaling and behavioural activation (Berridge, 2007; Robbins and Everitt, 2007; Salamone and Correa, 2012; Schultz, 2016), whereas dopamine in the dorsal striatum plays an important role in goal-directed behaviour and habit formation (Yin et al., 2008). Dopamine in the prefrontal cortex is generally assumed to underlie cognitive functions such as working memory and behavioural flexibility (Floresco, 2013; Robbins and Arnsten, 2009). These dopaminergic functions come about through two classes of receptors, the D1-like (dopamine D1 and D5 receptors) and the D2-like (dopamine D2, D3 and D4 receptors) (Seeman and Van Tol,

1994; Sibley and Monsma, 1992), that typically play overlapping, sometimes cooperative roles in the processes mentioned above.

Perhaps because of the diversity of functions subserved by forebrain dopamine, the pharmacology of dopamine in the study of social play has initially provided a quite complicated picture. That is, with a few exceptions showing increases in play after systemic treatment with the dopamine receptor agonist apomorphine (Beatty et al., 1984; Vanderschuren et al., 2008), systemic treatment with both dopamine receptor agonists (Niesink and Van Ree, 1989; Siviy et al., 1996) and dopamine receptor antagonists (Beatty et al., 1984; Holloway and Thor, 1985; Humphreys and Einon, 1981; Niesink and Van Ree, 1989; Siviy et al., 1996; Trezza and Vanderschuren, 2009) has been reported to reduce social play behaviour. Together, these findings suggest that during social play behaviour, dopamine neurotransmission functions at a subtle optimum, diversion of which disrupts the emotional and cognitive processes necessary for this behaviour. Alternatively, it may be the case that after systemic drug treatment, stimulation of social play behaviour as a result of increased dopamine signaling in one brain structure (for example the nucleus accumbens, see below) is negated or counteracted by increased dopamine transmission in another region (Cools and Robbins, 2004).

Previous work (Achterberg et al., 2014; Beatty et al., 1984; Beatty et al., 1982; Ferguson et al., 2000; Humphreys and Einon, 1981; Sutton and Raskin, 1986; Thor and Holloway, 1983; Vanderschuren et al., 2008), as well as more recent studies (Gamble et al., 2019; Ševcíková et al., 2020) have shown that treatment with psychostimulant drugs, such as cocaine, amphetamine, methamphetamine and methylphenidate, reduces social play. Importantly, the play-suppressant effects of psychostimulant drugs are comparable in male and female rats (Ferguson et al., 2000; Gamble et al., 2019; Sutton and Raskin, 1986). As the behavioural effects of these drugs are typically attributed to their stimulating effects on dopaminergic neurotransmission, these observation could add to the literature demonstrating that increases in forebrain dopamine lead to decreased social play. However, it should be borne in mind that the mechanism of action of psychostimulant drugs is not exclusive to dopamine, as these drugs increase noradrenergic and serotonergic neurotransmission as well. Indeed, pharmacological analyses have shown that the play-reducing properties of psychostimulant drugs such as amphetamine and methylphenidate rely on noradrenergic rather than dopaminergic signaling, through alpha-2 adrenoceptors (Achterberg et al., 2014; Vanderschuren et al., 2008), whereas the effects of cocaine on social play come about through an interaction of dopamine, noradrenaline and serotonin (Achterberg et al., 2014). Consistent, treatment with the selective dopamine reuptake inhibitor GBR12909 did not affect social play (Román et al., 2021a; Vanderschuren et al., 2008). More recently, the effects of the synthetic psychostimulant methylenedioxypyrovalerone (MDPV) on social play were reported (Schiavi et al., 2020). Treatment with MDPV was found to inhibit social play, but its effects were not identical to typical psychostimulant drugs. That is, unlike the effects of methylphenidate on social play (Vanderschuren et al., 2008), those of MDPV were subject to the development of tolerance after repeated treatment. In addition, the effect of MDPV on social play depended on stimulation of alpha2-adrenoceptors, comparable to methylphenidate and amphetamine, but unlike these latter drugs, on dopaminergic neurotransmission as well. Remarkably, the play-suppressant effect of MDPV is likely to occur through interactions between dopamine and noradrenaline (Schiavi et al., 2020).

As mentioned above, dopamine in the nucleus accumbens is important for certain reward-related processes, which makes it reasonable to implicate this system in social play behaviour. Indeed, stimulation of accumbens dopamine signaling by intracranial treatment with amphetamine or the selective dopamine reuptake inhibitor GBR-12909 increased social play behaviour (Manduca et al., 2016b). Conversely, intra-accumbens treatment with dopamine receptor antagonists – either non-selective, or selective for D1 or D2 receptors – reduced social play (Manduca et al., 2016b). These findings, implicating nucleus accumbens dopamine in social play behaviour, are supported by recent findings implicating post-weaning fluctuations in nucleus accumbens dopamine D1 receptor expression in social play, whereby higher D1 receptor expression resulted in an increase in social play (Kopec et al., 2018). Importantly, these D1-dependent developmental changes in social play behaviour occurred in male, but not in female rats. In addition to the nucleus accumbens, dopamine in the lateral septum has a role in social play (Bredewold et al., 2018). Thus, during social play behaviour, extracellular dopamine levels increased in the lateral septum in female, but not male rats. Intra-septal treatment with a dopamine receptor antagonist reduced social play in both male and female rats, whereby a higher dose was necessary in females. This latter finding suggests that baseline dopamine signaling supports social play in males, whereas increases in lateral septum dopamine facilitate social play in female rats. Interestingly, lateral septum dopamine appeared to modulate social play through interactions with vasopressin (see below; Bredewold et al., 2018). Although its importance for social play has yet to be directly demonstrated, prefrontal dopamine may be involved as well. Denying post-weanling rats the possibility to play during the weeks when this behaviour is most abundant results in altered dopamine signaling in the prefrontal cortex (Baarendse et al., 2013).

In line with the role of dopamine in motivational processes, we have previously shown that treatment with methylphenidate, cocaine and GBR-12909 increases responding for social play under a progressive ratio schedule of reinforcement (Achterberg et al., 2016a). The effect of methylphenidate on responding for play was blocked after pretreatment with a dopamine receptor antagonist. Importantly, consistent with findings described above, treatment with cocaine and methylphenidate reduced the actual expression of social play in this setup. The play-suppressant effect of methylphenidate was prevented by pretreatment with an alpha-2 adrenoceptor antagonist, demonstrating a double dissociation between the motivational properties of play – stimulated by methylphenidate through dopaminergic neurotransmission – and the performance of this behaviour – reduced by methylphenidate through noradrenergic neurotransmission (Achterberg et al., 2016a). Consistent with the notion that dopamine plays a more prominent role in motivation for rewards than in their pleasurable properties, changing dopamine neurotransmission hardly affected the development of social play-induced conditioned place preference. Thus, treatment with methylphenidate, cocaine or the dopamine receptor antagonist alpha-flupenthixol had no effect on social play-induced place preference. Intriguingly, treatment with GBR-12909 did block the development of place preference, for reasons yet to be elucidated.

With regard to interactions of dopamine with other neurotransmitter systems, previous work has implicated dopamine in the play-enhancing properties of indirect cannabinoid agonists, nicotine and alcohol (Trezza et al., 2009a; Trezza and Vanderschuren, 2008a, 2009). More recently, the nucleus accumbens was identified as a site of action for these interactions (Manduca et al., 2016b), since intra-accumbens treatment with a dopamine receptor antagonist prevented the play-enhancing effect of systemic treatment with the indirect cannabinoid receptor agonist URB597 or morphine (Manduca et al., 2016b). The latter effect is quite remarkable, since systemic dopamine receptor antagonist treatment did not influence the stimulating effect of morphine on social play (Trezza and Vanderschuren, 2008a), emphasizing the importance of detailed analysis of neural mechanisms of drug effects on play. As alluded to above, dopamine also interacts with vasopressin in the modulation of social play behaviour (Bredewold et al., 2018). Thus, intra-lateral septum treatment with a V1a vasopressin receptor antagonist prevented the increase in dopamine neurotransmission evoked by social play in female rats. Vice versa, treatment with apomorphine prevented the reduction in social play evoked by treatment with a V1a vasopressin receptor antagonist, demonstrating a bidirectional interaction between lateral septal dopamine and vasopressin in the modulation of social play.

In sum, there is a sizeable body of work implicating dopaminergic neurotransmission in social play (see Table 1). Whereas early pharmacological studies were somewhat inconclusive, later studies, directly targeting relevant brain structures or behavioural subcomponents of social play have painted a clearer picture. Most important are the identification of the nucleus accumbens and the lateral septum as sites of action through which dopamine influences social play, and the findings directly showing a role for dopamine in the motivation for play.

3.4. Noradrenaline neurotransmission

The brain noradrenergic system originates in the pontine locus coeruleus, that sends projections to a wide array of structures, including the brain stem, cerebellum, cortex, hypothalamus, thalamus, amygdala and hippocampus (Dahlström and Fuxe, 1964; Moore and Bloom, 1979; Ungerstedt, 1971). It has been implicated in a diversity of functions, such as arousal, attention, cognitive control, emotions and memory (Aston-Jones and Cohen, 2005; Berridge and Waterhouse, 2003; Floresco and Jentsch, 2011; Robbins and Arnsten, 2009). Brain noradrenaline exerts its effects through two classes of receptors, alpha and beta, of which the former are subdivided into alpha-1 and alpha-2 adrenoceptors (Bylund et al., 1994).

Previous systemic treatment studies have shown that noradrenergic neurotransmission, through alpha-1, alpha-2, as well as beta-adrenoceptors, modulates social play behaviour. Social play behaviour in these studies was found to be reduced after treatment with an alpha-1 adrenoceptor antagonist, alpha-2 adrenoceptor agonist, and a beta-adrenoceptor antagonist (Beatty et al., 1984; Normansell and Panksepp, 1985a; Siviy and Baliko, 2000; Siviy et al., 1994). Conversely, treatment with the alpha-2 adrenoceptor antagonists idazoxan and RX821002 was found to increase play (Siviy et al., 1990, 1994; Siviy and Baliko, 2000). Somewhat in contrast, treatment with a high dose of another alpha-2 adrenoceptor antagonist, yohimbine, had been found to reduce social play (Normansell and Panksepp, 1985a), but it cannot be excluded that the latter effect occurs through another mechanism than alpha-2 adrenoceptor antagonism, given the broad pharmacological profile of yohimbine. Since alpha-2 adrenoceptors are both auto- and heteroreceptors, the finding that their blockade increases play leaves open the possibility that increases in noradrenergic neurotransmission as a result of autoreceptor antagonism enhances social play. However, this latter possibility is less likely, since treatment with the noradrenaline reuptake inhibitor atomoxetine suppresses social play behaviour (Vanderschuren et al., 2008). This finding resonates well with the observations that psychostimulant drugs as such amphetamine, methamphetamine, methylphenidate and MDPV suppress social play (Achterberg et al., 2014; Beatty et al., 1984; Beatty et al., 1982; Humphreys and Einon, 1981; Schiavi et al., 2020; Ševcíková et al., 2020; Sutton and Raskin, 1986; Thor and Holloway, 1983; Vanderschuren et al., 2008). These drugs also block the reuptake – and in the case of amphetamine and methamphetamine stimulate the release of – noradrenaline. Further pharmacological analysis has demonstrated that the play-suppressant effects of amphetamine, methylphenidate and MDPV occurs through stimulation of alpha-2 adrenoceptors, since it was prevented by pretreatment with an otherwise inactive dose of RX821002 (Achterberg et al., 2014; Schiavi et al., 2020; Vanderschuren et al., 2008). As mentioned above, pretreatment with a dopamine receptor antagonist did not prevent the decreases in play evoked by amphetamine and methylphenidate, while it was effective against MDPV (Achterberg et al., 2014; Schiavi et al., 2020; Vanderschuren et al., 2008). Together, these findings indicate that general increases in noradrenergic neurotransmission, through blockade of autoreceptors or reuptake, reduce social play. Also, general reductions, as a result of alpha-2 agonism, suppress play as well, whereas stimulation of postsynaptic alpha-2 receptors increase play. The exact mechanism by which the latter effect occurs remains to be identified.

The neural mechanism by which noradrenaline reuptake suppresses play was investigated in a follow-up study, that revealed both cortical and subcortical substrates for this effect (Achterberg et al., 2015). Thus, infusion of methylphenidate or atomoxetine into the anterior cingulate cortex, infralimbic cortex, basolateral amygdala or habenula resulted in reductions in social play behaviour. In contrast, infusion of methylphenidate into the prelimbic cortex, orbitofrontal cortex, mediodorsal thalamus or nucleus accumbens shell did not affect social play (Achterberg et al., 2015). On the basis of the systemic drug studies described in the previous paragraph, it is reasonable to assume that the suppression of social play after intracranial administration occurs through stimulation of local alpha-2 adrenoceptors. Interestingly though, pretreatment with RX821002 into the anterior cingulate cortex, infralimbic cortex, basolateral amygdala or the habenula did not prevent the play-suppressant effects of systemic methylphenidate administration. Moreover, pretreatment with RX821002 into both the anterior cingulate cortex and the infralimbic cortex did not affect the effect of methylphenidate either (Achterberg et al., 2018). These findings suggest that noradrenaline reuptake reduces social play through several cortical and subcortical mechanisms in parallel, whereby prevention of the effect of methylphenidate in one single brain structure does not alter its effect on play.

In view of the importance of noradrenergic neurotransmission in cognitive and emotional processes, it is plausible that the changes in social play after systemic or intracranial treatment with noradrenergic drugs occur through changes in the cognitive or emotional aspects of play. This has been investigated in two setups. First, responding for social play under a progressive ratio schedule of reinforcement was found to be reduced after treatment with atomoxetine, that also resulted in reduces play during reinforced periods. In contrast, while methylphenidate treatment also reduced play, it enhanced responding for social play (Achterberg et al., 2016a). Importantly, the play-suppressant effect of methylphenidate was prevented by pretreatment with the alpha-2 adrenoceptor antagonist RX821002 but not a dopamine receptor antagonist, while the opposite was the case for responding for social play, revealing a double dissociation in the pharmacological modulation of play. This also suggests that the reduction in responding for social play after atomoxetine treatment – that does not affect dopaminergic neurotransmission – may be an indirect consequence of its suppression of social play behaviour itself. In the second setup, place conditioning was used (Calcagnetti and Schechter, 1992; Trezza et al., 2009b). In this paradigm, social play-induced conditioned place preference was not altered after atomoxetine pretreatment (Vanderschuren et al., 2016), suggesting that the positive emotional properties of social play do not depend on a noradrenergic mechanism of action. However, some of the cognitive mechanisms of social play-induced conditioned place preference, whereby repeated association of the positive emotions evoked by social play with a certain environment, causes this environment to elicit approach behaviour (Bardo and Bevins, 2000; Tzschentke, 1998, 2007). Thus, whereas treatment with the beta-adrenoceptor antagonist propranolol did not affect the acquisition or expression of social play-induced conditioned place preference, but it did block its reconsolidation after memory retrieval (Achterberg et al., 2012).

In sum, noradrenergic neurotransmission modulates social play behaviour through several mechanisms (see Table 1). Whereas general increases or decreases in brain noradrenaline signaling are not compatible with the optimal expression of social play, stimulation of postsynaptic alpha-2 receptors increases social play behaviour. Both cortical and subcortical mechanisms underlie the play-suppressant effects of noradrenaline reuptake inhibition. Last, whereas noradrenergic mechanisms do not appear to be involved in the pleasurable properties of social play, and perhaps only indirectly in its incentive motivational effects, they do affect some of the cognitive mechanisms by which social play experience influences later behaviour.

3.5. Serotonin neurotransmission

The brain serotonergic system originates in the brain stem raphe nuclei, a set of structures that provides the central nervous system with this neurotransmitter (Dahlström and Fuxe, 1964). These neurons project throughout the brain, including the frontal cortex, striatum, hypothalamus, thalamus amygdala and hippocampus (Azmitia and Segal, 1978; Dahlström and Fuxe, 1964). Serotonin exerts its effects through no less than seven main types of receptor (5-HT1–7), of which the 5-HT1, 5-HT2, 5-HT3 and 5-HT5 receptors can be divided into further subclasses, being implicated in a wide range of functions, including mood, cognition, sleep, circadian rhythm, pain and thermoregulation (Barnes et al., 2021).

Surprisingly, relatively little pharmacological work has been performed on the involvement of serotonergic neurotransmission in social play behaviour. Systemic treatment with drugs that enhance the extracellular concentrations of serotonin, such as the reuptake inhibitor fluoxetine or the releaser 3,4-methylenedixoymethamphetamine (MDMA) has been reported to reduce social play behaviour (Homberg et al., 2007; Knutson et al., 1996; Román et al., 2021a). Consistent, rats that are genetically deficient of the serotonin transporter protein display less social play (Homberg et al., 2007). An earlier study, using the relatively non-selective 5-HT2 receptor agonist quipazine and the 5-HT1B/D receptor antagonist methysergide found that treatment with both drugs decreased social play behaviour as well (Normansell and Panksepp, 1985b), an effect that was subsequently also reported after treatment with the 5-HT1A receptor agonist 8-OH-DPAT (Siviy et al., 2011). With regard to the underlying structure of social play, serotonin has been implicated in the asymmetry in play patterns that occur when established dominant and subordinate animals interact (Knutson et al., 1996; Siviy et al., 2011). In the first of these studies, treatment with fluoxetine exaggerated or diminished the asymmetry, depending on whether the subordinate or dominant animal was treated, respectively (Knutson et al., 1996). The other study (Siviy et al., 2011) reported that treatment with 8-OH-DPAT collapsed the asymmetry in play behaviour, regardless of whether the animal that showed the most or the least initiative to play was treated.

Serotonergic neurotransmission has been implicated in the development of social play behaviour. Studies that have investigated the protracted effects of prenatal and/or early postnatal exposure to selective serotonin reuptake inhibitors, as a proxy for maternal antidepressant use, have revealed that treatment with fluoxetine led to reduced levels of juvenile social play (Houwing et al., 2019; Olivier et al., 2011). After prenatal treatment with fluoxetine (GD11-birth), pinning but not pouncing was reduced (Olivier et al., 2011). Remarkably, exposure to fluoxetine during a period that encompassed both gestation and lactation (GD0-PND21) resulted in the reverse effect, i.e. a reduction in pouncing but not pinning, whereby both males and females were affected (Houwing et al., 2019). These results suggest that serotonergic neurotransmission during prenatal and early postnatal development determines the level of subsequent playfulness and the responsiveness to play solicitation. This is somewhat reminiscent of the studies described above that implicate serotonin in the ability to adjust behaviour to the level of playfulness of others (Knutson et al., 1996; Siviy et al., 2011). Early postnatal (PND8–21) treatment with another selective serotonin reuptake inhibitor, i.e. citalopram, also diminished juvenile social play (Khatri et al., 2014; Simpson et al., 2011). This effect was more pronounced in male, compared to female rats in one study (Simpson et al., 2011), but not the other (Khatri et al., 2014). Moreover, the effect of early life citalopram treatment on juvenile social play – in this study assessed as boxing/wrestling and following/chasing – depended on stimulation of either 5-HT1A or 5-HT1B receptors, since the effect of citalopram was mimicked by early treatment with a 5-HT1A receptor agonist, a 5-HT1B receptor agonist, but not prevented by pretreatment with a 5-HT1A or a 5-HT1B receptor antagonist (Khatri et al., 2014). Remarkably, in another study, treatment with fluoxetine from GD10-PND21 was found to increase social play, but in male animals only (Gemmel et al., 2019). However, in this latter study, the rats were tested during late adolescence/early adulthood (7–8 weeks of age), whereas in the other studies described here (Houwing et al., 2019; Khatri et al., 2014; Olivier et al., 2011; Simpson et al., 2011), the rats were tested as juveniles (in between PND24–35), suggesting that the protracted effects of prenatal/early postnatal exposure to selective serotonin reuptake inhibitors on social play may change with age.

In sum, the modest amount of work that has been done to study the role on serotonin in social play behaviour indicates that increases in serotonergic neurotransmission reduce social play behaviour, although the exact mechanisms remain to be identified (see Table 1). Given that serotonin exerts its effects through no less than 14 different receptor (sub)types (Barnes et al., 2021), there is still a lot to be investigated about the serotonergic pharmacology of social play behaviour. Interestingly, there is evidence that serotonin modulates the influence of dominance/subordination relationships on social play. Last, there is an emerging body of work implicating serotonin in the development of social play, showing that perinatal treatment with selective serotonin reuptake inhibitors inhibits later juvenile social play behaviour.

3.6. Vasopressin neurotransmission

Vasopressin is a short peptide consisting of nine amino acids, produced in the paraventricular and supraoptic nuclei of the hypothalamus, as well as in the bed nucleus of the stria terminalis and the medial amygdala. From its hypothalamic sources, it is transported to the pituitary for release into the peripheral bloodstream to regulate physiological variables such as blood pressure and water balance. The bed nucleus of the stria terminalis and the medial amygdala are the major sources of forebrain vasopressin, that modulates social behaviours, including social play (De Vries and Panzica, 2006; Donaldson and Young, 2008; Dumais and Veenema, 2016; Meyer-Lindenberg et al., 2011).

As far as we are aware, the first study that investigated the role of vasopressin on social play behaviour demonstrated that infusion of a vasopressin receptor antagonist into the anterior hypothalamus reduced social play in male hamsters (Cheng and Delville, 2009). Subsequent work by Veenema and colleagues revealed that vasopressin modulates social play behaviour in a sex-specific manner in rats. Thus, when a vasopressin receptor antagonist was infused intracerebroventricularly, it decreased social play behaviour in male rats, but increased it in females. Remarkably, the opposite pattern of effects was observed when the antagonist was administered directly into the lateral septum, i.e. an increase in males and a decrease in females (Bredewold et al., 2018; Veenema et al., 2013). This latter effect were most pronounced when the animals were tested in their home cages (Bredewold et al., 2014), and whereas intra-septal treatment with the vasopressin receptor antagonist enhanced both pinning and pouncing in male animals, it reduced only pinning – not pouncing – in females (Bredewold et al., 2018; Veenema et al., 2013). A subsequent histological study showed that after social play, the amount of active vasopressin cells, measured using Fos immunoreactivity, was not altered in the cell body regions of brain vasopressin, i.e. the paraventricular and supraoptic hypothalamus (Reppucci et al., 2018). An increase in cellular activity in vasopressin-containing neurons was found in the bed nucleus of the stria terminalis and the medial amygdala, implicating these structures in the influence of vasopressin on social play behaviour as well. Importantly, the increase in activity of vasopressin cells in the above regions was more pronounced in females (Reppucci et al., 2018).

Vasopressin signaling has also been implicated in the development of social play. During the ontogenetic emergence of social play, i.e. on postnatal days 18–21, Paul et al. (2014) found that vasopressin expression in the bed nucleus of the stria terminalis was negatively correlated with the amount of play displayed, whereas a positive correlation was found in the paraventricular hypothalamus. Again, these effects were sex-specific, as they were found in male rats only. On the

Table 2
Pharmacological and genetic models, early life manipulations and environmental factors that influence play in rats.

Procedure/ manipulation	Action	Target	Strain[a]	sex	Dose	Treatment	Timing treatment[b]	Age play tested (PND)	Isolation procedure (hrs)[c]	**Play observation time (min)[d]**	**Type of partner[e]**	**Effect on play[f]**	**Reference**
Valproic Acid (VPA) model													
Valproic Acid	mood stabilizer, anti-epilepticum	increasing GABA neurotrans-mission by inhibiting degradation enzyme	le	females	800 mg/kg	VPA	GD 12.5	29 and 34	24	10	am, sh, st	- but microstructure altered	Raza et al. (2015)
			w	males	500 mg/kg	VPA	GD 12.5	35	3	15	ut	↓ pouncing	Servadio et al. (2016)
			w	males	500 mg/kg	VPA	GD 12.5	35	3	15	ut	↓ pouncing, ↑ partial rotation	Melancia et al. (2018)
			w	males	400 mg/kg	VPA	GD 12.5	30	PND21–30	10	nis	↓	Cezar et al. (2018)
			w	males	500 mg/kg	VPA	GD 12.5	35–40	3	15	ut	↑ partial rotations	Schiavi et al. (2019)
			sd	both	500 mg/kg	VPA	GD 12.5	31–32	2.5	10	u, wm	↓ males, - females	Gzielo et al. (2020)
			w	males	600 mg/kg	VPA	GD 12.5	30	-	15	u, st	↓	Roman et al., 2021
Genetic models													
Shank3-knock out	synaptic scaffolding protein located in the postsynaptic density of glutamatergic synaps		sd	both				32–45	0.5	10	u, sm, am	-	Berg et al. (2018)
Nrxn1α-mutation	synaptic function	synaptic protein	Nrxn1tm1Sage-sd	both	haplo or biallelic mutation	none		27–30	-	10 for 4 days	sm, u, sg	males, ↓ KO	Kight et al. (2021)
Cacna1c haploinsufficiency	risk gene neurodevelopmental disorders		sd	both				32–34	24 for 3 days	5	sm, sg, am, u	females ↑, - males	Kisko et al., (2018, 2020); 2021
Fischer-344 rat	disrupted vesiclar release of dopamine		F344	males				36–40	24	4 hrs and 24 hrs	ss	↓	Siviy et al., 2020
(Maternal) Immune Activation													
MIA + post natal hypoxia		white matter	w	both	100 μg/kg	LPS (lipopolysaccharide), on PND4 140 min exposure to 8% oxygen		28–32	2.5	15	u, wm, sm, st	↓ both (pouncing), ↓ pinning (males)	Van Tilborg et al. (2018)
Allergic challenge during pregnancy	increase in immunoglobin E		sd	both	1 mg/1% per 50 μl per nare	Ovalbumin	2x prior to pregnancy/ GD 15	28–38	-	10 for 5 days	group of 6/7 mixed sex and treatment, consisting of at least 3 males	↓	Breach et al. (2021)
MIA			sd	both	5 mg/kg	poly(I:C)	GD 15	32–35	2.5	10	sm, st, u	↓ males, - females	Gzielo et al. (2021)
MIA			w	both	500 μg/kg	LPS (lipopolysaccharide)	GD 16	33	3.5	10	u, sm, st	↓ males, - females	Vitor-Vieira et al. (2021)
postnatal immune challenge			sd	both	0.1 mL of 1 × 10^6 CFU/100 μg/kg	E. coli/LPS	PND4/ PND25	28	3	60 in 4 increments	ut, u, sm, am, wm	↑	Turano et al. (2021)

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Table 2 (*continued*)

Procedure/ manipulation	Action	Target	Strain[a]	sex	Dose	Treatment	Timing treatment[b]	Age play tested (PND)	Isolation procedure (hrs)[c]	**Play observation time (min)[d]**	**Type of partner[e]**	**Effect on play[f]**	**Reference**
Early life adversity (ELA)													
Exposure to predator odour		brain development	sd	both	5 min/day		PND1–3	27–32	-	10 for 5 days	u, sm, st	↓ males, ↑ females	Stockman and McCarthy (2017)
Daily maternal separation		brain development	w	both	15 min or 6 hrs		PND1–21	33–35	3.5	15	u, st, sm, wm	↑ (6 hrs group)	Lundberg et al. (2017)
Maternal separation and complex housing		brain development	w	both	24 h		PND 3/ PND26-end (complex housing)	33–42	3 or 24	15	u, wm, sm, st	↑ (complex housing)/ - (maternal separation)/	Kentrop et al. (2018)
ELA	short-acting genotoxin and antiproliferative agent	brain development	sd	both	22 mg/kg	methylazoxymethanol (MAM)	GD17	31–32	2.5	10	u, sm, st	↓	Potasiewicz et al. (2019)
Exposure to predator odour		brain development	sd	both	5 min/day		PND1–3	25–30	-	5 during 5 days	10 mixed-sex, mixed littermates/ non littermates per experimental condition	↓	Cuarenta et al. (2021)
Stress exposure (variable ultrasounds)		brain development	w	both			GD1-GD21	35	3.5	15	sm, u	↓ males	Abramova et al. (2021)
Limited bedding ELA		brain development	sd	both			PND2–14	34	0.17	10	ut, am, sm	-	Granata et al. (2022)
Adversity during juvenile and adolescent period													
Repeated exposure to restraint and effect κ-opioid receptor			sd	both	90 min/day, 5x		PND24–29	28, 35 and 70	0.5	10	sm, u, ut	↑ (juvenile animals), no effect adolescent or adult animals	Varlinskaya et al. (2018)
		κ-opioid receptor agonist			0.1–0.4 mg/kg	U-62066						↓	
Peripubertal stress			w-Han	males	7x open field (5 min), exp. to fox odour (25 min), exp. to an elevated platform (25 min)		PND28–42	45	3.5	15	wm, st, u	↑	Papilloud et al. (2018)
Paternal stress and adoption			w-Han	both	paternal: stress 25 min on PND34–36–42/ PND28–30, 40 offspring adoption PND1			45	3.5	15	st	↑ ↑	Zutshi et al. (2021)
Environmental pollutant exposure													
Prenatal phtalate and maternal high fat diet			le	both	0, 200, or 1000 mg/kg/d	phtalate mixture	G0-PND10	between 32 and 40	1 on 4 consequtive days	20 on 4 consequtive days	u/f, am, sm, st	↓ at 200 mg/ kg/day	Kougias et al. (2018)

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Table 2 (*continued*)

Procedure/ manipulation	Action	Target	Strain[a]	sex	Dose	Treatment	Timing treatment[b]	Age play tested (PND)	Isolation procedure (hrs)[c]	Play observation time (min)[d]	Type of partner[e]	Effect on play[f]	Reference
					45% kcal fat, 20% kcal protein, 35% carbohydrate	High fat diet	G0-PND01					-	
Bisphenol A (BPA) and high fat diet	endocrine disruptor		le	both	0, 40, or 400 μg BPA/kg/day	BPA	G0-PND10	26–40	1 on 4 consequtive days	20 on 4 consequtive days	sm, st, u	↓	Wise et al., 2019
					45% kcal fat, 20% kcal protein, 35% carbohydrate	High fat diet	G0-PND01					-	
Particulate Matter (PM)			sd	males	200 μg/m3 (measured as PM2.5), 5hrs/ day, 5days/ week	Boston highway tunnel exhaust plenum	~G0-PND23	between 32 and 40	-	60	f, sm, st	↓	Nephew et al. (2020)
Glyphosate (GHB)	Pesticide		w	both	50 mg/kg	GHB	G0-PND22	28–32	3.5	10	sm, u, wm	↓	De Oliveira et al. 2022
Other pre-, peri- or postnatal treatments affecting social play													
Childhood concussion	mild traumatic brain injury (mBTI)		sd	both	Between ears		PND30	37	24	10	f, sh, sm	- males ↓ females	Mychasiuk et al. (2014)
			le	both	2 mm below skull location		PND27	30–31	24	10	vt, u	-	Dyck and Ivanco (2018)
Perinatal asphyxia with water immersion			sd	males	15 degrees celsius	water immersion	20 min directly after birth	37–38	24	15	am, sm, u, vt	↓	Vázquez-Borsetti et al. (2019)
					37 degrees celsius	water immersion	20 min directly after birth					↓	
					15/37 degrees celsius/10 degrees Celsius	water immersion/ room	20 min directly after birth/ directly after asphyxia					↑ compared to asphyxia/- compared to control group	
Overweight mother and litter size of mother			w	both	12/4 pups (50% male/ female) per litter			28–32	3.5	10	sm, u, wm	↓ compared to normal mother and/or normal litter	De Novais et al., 2021
Methamphetamine	DA/NA/5HT releaser		w	both	5 mg/kg	Methamphetamine	GD1–11 or GD12–22	30	16	10	st, sm	↓ males, - females	Ševcíková et al. (2020)
Prenatal alcohol exposure			sd	both	6.37% v/v	Ethanol	GD1-PND21	30 for females, 36–38 for males	overnight	10	sm, u	↓	Holman et al. (2019)
Environmental enrichment			w	both		Environmental enrichment	PND21–71	35	4	15	sm, st, u	-	Cutuli et al., 2018

[a] W: Wistar, sd: Sprague-Dawley, le: Long-Evans, F344: Fischer 344

[b] GD: gestrational day, PND: post-natal day

[c] Hrs: hours, PND: post-natal day

[d] Min: minutes

[e] am: age matched, f: familiar, lm: littermate, nis: non-isolated stimulus animal, sg: similar genotype, sh: socially housed, sm: sex matched, ss: similar strain, st: similar treatment, u: unfamiliar ut: untreated, vt: vehicle treatment, wm: weight matched, yp: younger partner

[f] ↑: increase, ↓: decrease, - no effect

other hand, it was found that social play was reduced throughout development in both male and female Brattleboro rats, that lack vasopressin due to a mutation in the vasopressin gene (Paul et al., 2016; Schatz et al., 2018).

In sum, vasopressin neurotransmission plays an important role in the modulation of social play, both during its development as well as its juvenile expression (see Table 1). Importantly, the role of this neuropeptide in social play behaviour is sex-specific, and involved brain structures including the anterior and paraventricular hypothalamus, lateral septum, bed nucleus of the stria terminalis and the medial amygdala.

3.7. Summary

In this section, we have summarized previous and more recent work on the neural underpinnings of social play behaviour in rats. As we hope that the reader will appreciate, the neurobiology of social play continues to be an active field of research, and work in the last two decades has started to paint a more fine-grained picture of the playful social brain. For example, following up on earlier work (Normansell and Panksepp, 1990), recent data further implicate opioid neurotransmission in the positive emotional properties of social play (Achterberg et al., 2019), whereas dopamine has been shown to play an important role in the motivation to play (Achterberg et al., 2016a). Furthermore, noradrenergic neurotransmission is involved in certain cognitive aspects of social play (Achterberg et al., 2012), serotonin in how dominance relationships influence play (Knutson et al., 1996; Siviy et al., 2011), and vasopressin in the sex differences in social play (Bredewold et al., 2018; Veenema et al., 2013). The development of social play behaviour, then, has been shown to be under the influence of cannabinoid (e.g. Trezza et al., 2008; Argue et al., 2017), serotonin (e.g. Olivier et al., 2011; Khatri et al., 2014) and vasopressin (Paul et al., 2016; Schatz et al., 2018) signalling. Clearly, our knowledge of the neural basis of social play behaviour is still far from complete. In addition to employing sophisticated contemporary neuroscience tools such as optogenetics and chemogenetics to reveal the neurobiology of social play in more detail, there are several important overarching questions that deserve our attention (see also 'The future: outstanding questions', below), such as to what extent the mechanisms at hand are specific to social play behaviour, or that they also underlie other (social) behaviours. For example, given the well-established role of opioids and dopamine in the positive emotional properties of rewards such as palatable food, sex and substances of abuse (e.g. Le Merrer et al., 2009; Salamone and Correa, 2012), it should be no surprise that these are also important for the pleasurable and motivational aspects of social play. By which neural processes cognitive and perceptual aspects of social play come about, such as identification of potential play partners, properly interpreting social signals, continuously monitoring behaviour of oneself and others and adjusting behaviour accordingly, remains to be shown. That said, existing work has already provided important clues for roles of noradrenaline (Achterberg et al., 2012), serotonin (Knutson et al., 1996; Siviy et al., 2011) and frontal cortical mechanisms (Pellis et al., 2006; Bell et al., 2009; Bijlsma et al., 2022, 2023) in these aspects of the behaviour that are more likely to be specific for social play.

4. Embedding social play behaviour in neurobehavioural investigations

The studies summarized above have for the most part explicitly studied the neural mechanisms of social play behaviour. Interestingly, while some research groups focus on understanding this behaviour from a (neuro)-evolutionary or a neurobehavioural perspective trying to understand the function of social play behaviour, in recent years, an increasing number of studies include social play as an behavioural measure to investigate manipulation effects in the perinatal, juvenile or adolescent period in the life of rats as part of a test-battery ranging from birth to adulthood. Studies modelling neurodevelopmental disorders of which a core symptom is that social behaviour is affected, such as Autism Spectrum Disorder (ASD), Attention-Deficit Hyperactivity Disorder (ADHD) and schizophrenia have been incorporating social play behaviour analysis as an early measure for social deficits (see Table 2).

4.1. The valproic acid model of ASD

Valproic acid (VPA) is a mood stabilizer and an anti-epileptic drug. Clinical studies over the past four decades have shown that exposure to VPA *in utero* is associated with birth defects, cognitive deficits, and increased risk of ASD (for reviews see Roullet et al., 2013, Mabunga et al., 2015). In both mice and rats, *in utero* exposure to VPA recapitulates many of the structural and behavioural abnormalities that can be observed in individuals with ASD such as an excitatory to inhibitory (E/I) imbalance, impaired social behaviour and communication as well as restricted and repetitive behaviour. The model is therefore thought to be instrumental in characterizing relevant pathways of developmental dysregulation and aids in developing potential therapeutic candidates addressing the core symptoms of neurodevelopmental disorders.

Gzielo et al. (2020) showed that male VPA-exposed rats displayed reduced total play duration, pouncing and pinning. In contrast, female VPA-exposed rats did not show these reductions in play parameters during the juvenile period and these sex-differences were still present in adult social behaviour. In line with the absence of a reduction in social play in female rats, Raza et al. (2015) found the microstructure of play to be altered, i.e. VPA exposed females were less likely to use body contact promoting defensive tactics and these rats made less USVs. In another study, VPA-exposed rats display atypical patterns of social play behaviour compared to control animals, as rats prenatally exposed to VPA play less and respond differently to play solicitation, i.e. VPA exposed animals use partial rotation and evasion, instead of pinning (Servadio et al., 2016; Melancia et al., 2018). Melancia et al. (2018) investigated social, emotional and cognitive behaviour in VPA-exposed Wistar rats of both sexes. Females played less than males but both sexes showed the altered response to play solicitation after VPA exposure. General social interest in the form of social exploration was not affected by prenatal VPA treatment. The differences in results compared to Gzielo et al. (2020) can be explained by the slight differences in the testing procedures in both labs and the ethogram followed for assessing social play behaviour in these rats. Next to the effect of the VPA on social play in both sexes, systemic treatment with the anandamide hydrolysis inhibitor URB597 normalized the increased partial rotations in VPA treated females. This is in line with Servadio et al. (2016), who found that URB597 rescued the reduced responding to play solicitation in VPA-exposed males. These results not only point to a role for endocannabinoid neurotransmission in developing therapeutic targets for VPA-induced ASD-like behaviour but also add to our knowledge on the involvement of the endocannabinoid system in modulating social play behaviour.

From the same lab as the previous study, Schiavi et al. (2019) confirmed that VPA-exposed male rats did not differ in the number of play initiations (pouncing) or complete rotations (pinning) but showed an increased number of partial rotations. Also, the response to pouncing was lower in VPA-treated animals. Additional tests showed that VPA treated male rats are less social in the 3-chamber task and displayed a lower discrimination index towards a novel juvenile animal. Animals were also tested for reward-related behaviours to investigate whether it is reward processing in general or social reward in particular that is affected by prenatal VPA treatment. VPA-exposed rats did not differ from control rats in amphetamine-induced hyperlocomotion, sucrose preference and socially-induced conditioned place preference (sCPP). Analyzing dopamine receptors in the brain during adolescence revealed no differences between VPA-exposed and control rats in D1 dopamine receptor expression in prefrontal cortex, nucleus accumbens, dorsal striatum and hippocampus. In contrast, D2 dopamine receptors were

increased in the nucleus accumbens of VPA-treated rats. In adulthood, an increase in dopamine D1 and D2 receptors was found in the nucleus accumbens of VPA treated rats. Also, an increase in D1 receptors was observed in the hippocampus of VPA treated rats. In nucleus accumbens medium spiny neurons of VPA-exposed animals, altered neurophysiological properties were observed, i.e. a higher firing probability in conditions of normal synaptic excitation. The authors suggest that the social deficits displayed by VPA-exposed rats in the course of development may arise from dopaminergic dysfunctions in both the nucleus accumbens and hippocampus but that social reward processing in general was not altered.

Exploring dopaminergic and serotonergic neurotransmission as potential targets for treatment of ASD, Román and colleagues (2021b) investigated the effect of the antipsychotic, antidepressant, and antimanic compound cariprazine, a dopamine D3-preferring receptor and serotonin 5-HT1a receptor partial agonist, in the VPA model. This compound was compared to the known antipsychotics risperidone (a 5-HT2 receptor and dopamine D2 receptor antagonist) and aripiprazole (a partial agonist at dopamine D2 receptors, a partial agonist at 5-HT1A receptors, and an antagonist at 5-HT2A receptors). The authors showed that VPA reduces both the frequency and duration of social play and social interaction. Treatment with the highest dose of cariprazine (0.1 mg/kg) restored the frequency and duration of play and social investigation to the levels of non-VPA treated rats whereas risperidone and aripiprazole did not have an effect on social play or social exploration. Hyperactivity, stereotypic behaviour, behaviour in the social approach-avoidance test and social recognition memory were rescued with all three compounds. This suggests that cariprazine has unique properties, via dopamine D3 receptors, to rescue social (play) behaviour at an earlier age compared to the other anti-psychotics.

Cezar et al. (2018) investigated whether the impairments in social play, ultrasonic communication and cognitive abilities found after VPA treatment could be alleviated with zinc supplementation (directly after VPA treatment pre-birth), since VPA causes reduced availability of zinc to the fetus. The authors found that VPA-induced impairments (reduced social play, decreased USVs, rigidity in the T-maze) could be alleviated with zinc supplementation. However, it should be noted that zinc supplementation without VPA treatment had the same effect as VPA treatment in the frequency of pins, albeit less pronounced. Interestingly, the authors also found that VPA treatment resulted in reduced expression of tyrosine hydroxylase (the rate-limiting enzyme of dopamine biosynthesis) in the striatum which was unaffected by zinc supplementation.

These studies show that VPA treatment either reduces social play or the response to play solicitation. Furthermore, they add to the existing knowledge on the involvement of dopamine and endocannabinoids in social play behaviour, possibly pointing to an altered social motivational state in VPA exposed rats.

4.2. Genetic models of ASD

Alongside the VPA model, rat models have been developed for assessing the contribution of specific risk genes in neurodevelopmental disorders. Loss-of-function mutations in the synaptic protein neurexin1α (NRXN1α) are associated with several neurodevelopmental disorders, including ASD, schizophrenia, and ADHD. Kight et al. (2021) investigated several cognitive and social behaviours, including social play behaviour in wildtype, heterozygous and homozygous knock-out rats of this gene. The authors found reduced pounces, pins, boxes and total play events in male but not female Nrxn1α-KO rats. Heterozygous male rats were not different from wildtype males, except that they boxed more compared to wildtype females. Next to less play, KO animals also showed alterations in other (social) behaviours starting from a young age. KO pups emitted less ultrasonic vocalizations when separated from the mother. In adulthood, KO males but not females showed increased locomotor activity and less prosocial helping. KO animals also showed a reduced latency to care for unfamiliar pups and deficits in social discrimination.

Another gene of interest for several neuropsychiatric disorders, including schizophrenia, bipolar disorder, major depressive disorder and ASD is CACNA1C. This gene encodes the alpha-1 C subunit of the voltage-dependent L-type calcium channel Cav1.2 involved in neuronal excitability and synaptic plasticity (Zamponi, 2016). Kisko et al. (2020) showed that the gene plays an important role in the development of social behaviour and communication. Cacna1c haploinsufficiency in rats resulted in higher social play levels in females, but did not alter the time spent on social play in males compared to wildtype rats. Ultrasonic vocalizations (USVs) are an expression of the affective state of the animal as well as a means of communication in rats (Wöhr, Schwarting, 2013; Burke et al., 2022). In addition to the increased play in females, males partially lacking the Cacna1c gene emitted less 50-kHz USVs, whereas females did not alter their USV emissions (Kisko et al., 2018, 2020).

Berg et al. (2018) investigated the effect of the autism-related gene Shank3, that codes for a synaptic scaffolding protein, on social behaviour and communication in rats, including social play. Social play (related) behaviour parameters, i.e. boxing, pushing under or crawling over, pinning, and pouncing were not affected by genotype or sex. KO male rats showed reduced social investigation parameters (allogrooming, anogenital sniffing) compared to females. Next to the differences in social play behaviour, 50-kHz induced social approach behaviour was reduced in shank3-KO male rats compared to heterozygous, wildtype and their female counterparts.

These studies show that deficits in recognized risk genes for neurodevelopmental disorders affect social behaviour early in the post-natal development of rats. When social play behaviour is incorporated in longitudinal rat studies, alterations in complex social behaviour can be recognized in the juvenile, adolescent and adult phase to provide a more complete social behavioural profile (see Table 2). Aside from the translational value of longitudinal studies, this allows for a broad window of timepoints as targets for treatments.

Behavioural consequences of a genetic predisposition may also be influenced by changing the postnatal environment in rats. Siviy (2020) assessed the effect of cross fostering on social play behaviour in Fischer-344 rats. This particular inbred strain is known to be less playful and has disrupted vesicular release of dopamine (Siviy et al., 2011). Fischer 344 rats showed less nape contacts (pounces) and were less likely to respond to a nape contact with a complete rotation (pin) than were Lewis control rats. Cross-fostering Fischer 344 rats with mothers of a more playful strain (i.e. Lewis) did not alter absolute levels of play in Fischer 344 rats, which led the author to conclude that differences in social play between these two strains are not necessarily due to altered maternal care or early post-natal environment but may be driven by genetic factors (Siviy et al., 2003; Siviy et al., 2017). In a follow-up experiment, housing Fischer 344 rats together with two other Fischer 344 or Sprague-Dawley rats induced alterations in the response to a nape attack (i.e. pounce). Fischer 344 rats housed with more playful rats did not increase absolute play frequency but the probability to rotate to a pin increased significantly. Siviy (2020) concluded that, although playing at low levels, Fischer 344 rats alter their response to pounces according to the type of play displayed by their cage-mates. These results increase our existing knowledge on the involvement of dopamine in modulating social play. The next step would be to investigate whether these animals have an altered motivation to engage in social play. In addition, besides uncovering the basic functioning of the brain, these results provide us clues of how play behaviour may be adjusted by the environment when a genetic predisposition alters this behaviour.

4.3. Immune activation

Maternal immune activation (MIA) or maternal inflammation has been found to increase the risk for ASD, ADHD, and schizophrenia in offspring (for review see Gumusoglu and Stevens, 2019). Breach et al.

(2021) used MIA to characterize social, emotional, and cognitive behaviour throughout the lifespan as well as the levels of mast cells and microglia in the perinatal period in male and female offspring. MIA resulted in reduced play behaviour and chasing in both male and female offspring. In addition, allergic challenge with ovalbumin did not alter passive or active social investigation, but maternally challenged offspring were less social compared to vehicle-offspring. Allergic inflammation decreased anxiety-like behaviours in the open field but not in the elevated plus maze. Cognitive performance as measured in the attentional set shifting task did not differ between the sexes. Maternally challenged offspring took more trials to reach criteria after the reversal and in the extradimensional shift phase suggesting possible impairments in cognitive flexibility. Microglia amounts in brain regions involved in social play behaviour (medial prefrontal cortex, amygdala, hippocampus and the basal ganglia) in the fetal and postnatal brain were altered due to maternal immune activation and changes in microglia composition and form were occasionally sexually dimorphic.

Using poly(I:C) to induce MIA, Gzielo et al. (2021) investigated its effect on social play-induced USVs in the offspring. MIA reduced pins, percentage of play responses and total play duration without affecting the total number of pounces in males whereas females were unaffected. The number of USVs emitted during social play was reduced and their characteristics were altered in animals from MIA-mothers. In addition, Poly(I:C) exposure increased locomotor activity and stereotypic-like behaviour in both males and females. These results show that maternal immune activation with poly(I:C) impairs aspects of social play behaviour, communication characteristics and affects stereotypic behaviour in a way that resembles ASD.

MIA induced with the endotoxin lipopolysaccharide (LPS) resulted in altered play behaviour in the offspring as well. Vitor-Vieira et al. (2021) tested the core symptoms of ASD in several behavioural tasks (i.e. communication, homing behaviour, social play behaviour, stereotypy, anxiety and locomotion). They found that males from mothers that underwent immune activation during gestation showed less social play which was not the case in females. The males showed impairments in communication and cognition compared to MIA-females. The expression of the neural activity marker c-fos was assessed in brain regions associated with social play behaviour, emotion regulation and cognition, and reduced c-fos expression in the anterior cingulate cortex and nucleus accumbens shell and increased c-fos expression in basolateral and basomedial amygdala was found in offspring from LPS-treated mothers.

Using a slightly different approach, Carbone et al. (2023) also found reduced play behaviour in male Wistar rats from LPS-treated mothers. In addition, LPS caused alterations in social communication in the pups, while maternal care and pup homing behaviour were unaffected. In adolescence, next to the reduction in play, rats from LPS-treated mothers showed less sociability in the 3-chamber task but no stereotypic-like behaviour. However, in adulthood, social discrimination and sociability deficits were apparent as well as stereotypic-like behaviours. No increased anxiety was observed in these animals. Flow cytometric analysis was performed to compare plasma levels of inflammatory markers in both adolescence and adulthood. The authors found no differences in adolescence whereas in adulthood the pro-inflammatory cytokines IFN-γ and MCP-1 as well as the anti-inflammatory cytokine IL-13 were enhanced in the LPS treated offspring.

Using a double hit model for white matter injury (WMI) in prematurely born children, van Tilborg et al., (2018) used prenatal immune activation in combination with post-natal hypoxia to induce WMI. Several behavioural paradigms for motor coordination, cognitive functioning, anxiety-like and ASD-like behaviour including social play behaviour were assessed. Social play behaviour was specifically reduced in male WMI rats whereas female play behaviour was unaltered. In addition, decreased motor coordination and increased anxiety-like and repetitive behaviour were observed without effects on simple cognitive tasks. These data reinforce the idea that WMI could lead to an ASD-like behavioural phenotype.

Turano et al. (2021) tested whether immune dysregulation at a young age affected social behaviour in male and female rats. Social interaction was slightly reduced by neonatal infection regardless of LPS administration. Neonatal infection regardless of whether animals were later again challenged with LPS significantly increased the expression of play in male and female animals but had no effect on how the non-treated stimulus animal played with them. Neonatal infection, in combination with juvenile LPS-administration, significantly increased inflammatory responses in several brain regions involved in social play (i.e. hippocampus, amygdala, medial prefrontal cortex and anterior cingulate cortex).

Immune challenges during gestation reduce social play behaviour whereas an early postnatal challenge seems to increase the amount of social play (see Table 2). Together, these studies identify social play as an early marker for neurodevelopmental disorders as a consequence of maternal immune activation alone or in combination with environmental factors. In addition, the timing of immune challenge seems to be important for the social behavioural outcome thereby providing clues on how brain development impacts on social play behaviour.

4.4. Early life adversity

Similar to maternal immune activation, early life adversity (ELA) increases the risk for neurodevelopmental disorders that involve disrupted social functioning. Abramova et al. (2021) investigated exposure to stress during pregnancy by exposing rats to variable frequency ultrasounds as a proxy for psychological stress instead of physical stress. These sounds have a frequency that resemble distress signals in rats. Animals from ultrasound-exposed mothers played less, which was especially evident in males. Social exploration was similar between groups suggesting equal social interest. Prenatal exposure to variable frequency ultrasounds also increased anxiety in the open-field test and changed social preferences in the social novelty test.

In the study of Potasiewicz et al. (2019) an animal model for schizophrenia was used where pregnant dams were treated with methylazoxymethanol acetate (MAM). MAM is a short-acting genotoxin and antiproliferative agent, disrupting brain development. Social play frequency and duration was significantly reduced in both MAM-treated males and females. MAM treated animals also had significantly longer latencies to engage in play. USV emitted in various tests (pup isolation, homing behaviour, tickling and social play behaviour) showed subtle differences in call characteristics. On average, MAM rats emitted more flat calls and less frequency modulated calls.

Granata et al. (2022) used the limited bedding ELA model and assessed social behaviour at different timepoints as well as oxytocin receptor and vasopressin receptor 1a mRNA expression in the mPFC. Limited bedding did not affect social play frequencies and durations. Total emitted USVs and the proportion of complex calls were lower in pups of both sexes after limited bedding. In the prefrontal cortex, oxytocin receptor mRNA was enhanced whereas vasopressin receptor 1a mRNA was unaffected in limited bedding rats.

Cuarenta et al. (2021) exposed pups to predator odour and assessed social play behaviour in adolescence. They also investigated how the retrotransposon Line1 and its two open reading frames (Orf1p and Orf2p) alters gene expression in the amygdala, hippocampus and prelimbic cortex. The authors found that early life stress resulted in lower play levels. They also found a negative correlation between social play levels and Line 1 Orf 1 DNA copy number in the amygdala whereas no effects were found in the hippocampus or prelimbic cortex. Thus, adverse early life experiences such as exposure to predator odour creates alterations in Line1 levels within the juvenile brain suggesting that stressful experiences early in life have the capacity to change the genome via altered retrotransposon activity. The lower levels of play may be specifically caused by Line1 genomic changes within the amygdala.

Predator odor exposure of neonatal male and female rats resulted in

reduced USV emission and more freezing behaviour in both male and female pups whereas only males showed elevated corticosterone levels post-exposure (Stockman and McCarthy, 2017). Predator odour exposure reduced the time spent playing as well as its frequency in males but increased these measures in females. No effects of predator odour exposure on anxiety measures in the open field, elevated plus maze or light-dark box were found. When retested in adulthood, social and anxiety-like behaviours were not affected by early predator odour exposure. These data suggest that while social play behaviour is affected in a sex-specific way by neonatal predator odor exposure, this does not necessarily translate into an anxious phenotype. Whether it makes some animals more vulnerable to exposure of other stressors remains to be investigated.

Lundberg et al. (2017) studied the effect of daily maternal separation on social play behaviour and stress responsivity. Regarding social play behaviour, males separated for 6 h showed a higher frequency of pinning than males separated for 15 min, whereas the frequency of pouncing was not different between rearing conditions. There were no differences in latency to pin or pounce. Rearing condition did not result in altered corticosterone levels, but 6 h-separated males had higher baseline and post-test levels of corticosterone than 15 min-separated males.

Kentrop et al. (2018) tested the effect of maternal deprivation and complex housing on the social behaviour of adolescent and adult rats. An enriched environment was chosen as a potential intervention to counteract the effects of early life stress. Male and female rats were deprived from their mother or left undisturbed. Complex housing started 5 days after weaning and consisted of housing 10 same-sex conspecifics in large enriched cages until the end of the study. Social play behaviour in adolescence was tested after 3 h or 24 h of social isolation prior to testing. After 3 h of social isolation, no effects of maternal deprivation were found. Complex-housed animals played less and huddled together more (social rest) while social exploration was unaffected. After 24 h of isolation, complex housed males played more, pinned faster and also showed more social exploration compared to standard housed males. Female play behaviour was unaffected by maternal deprivation and housing condition, although complex housed females pinned faster and showed increased social exploration compared to standard housed females. As adults, in standard housing conditions, maternal deprivation did not affect social interest in the 3 chamber task but deprived animals had difficulty in social discrimination. Complex housed males showed less social interest but a comparable social discrimination as standard housed animals whereas adult female social behaviour was unaffected by complex housing. The authors conclude that while maternal separation does not affect social play behaviour, complex housing induces a distinct behavioural phenotype in adolescence and adulthood. In addition, males were more affected by the treatments than females.

Taken together, early life adversity in several forms can subtly affect social play behaviour, with reductions, no effects or increases in social play compared to the control conditions (see Table 2). This diversity in behavioural outcomes highlights the importance of the impact of early life adversity (and the experimental set-up) on social play behaviour but also how animals cope with this.

4.5. *Adversity during the juvenile and adolescent period*

Zutshi et al. (2021) investigated the transgenerational effect of paternal stress and early adoption on social behaviour, social memory and anxiety of the offspring. Rats were exposed to an elevated platform or predator odor, either exclusively or sequentially after which they were mated in adulthood. Shortly after birth, half of them were cross fostered while the other half stayed with the original mother. Play behaviour was enhanced in animals that had been exposed to stress prenatally. In addition, being raised by a prenatally stressed mother resulted in an increase in pounces but no additive effect of postnatal stress exposure was observed. Aggression towards an intruder, social investigation and discrimination in the 3-chamber task towards juveniles, social memory, shock probe and depression-like behaviour in the forced swim task were also investigated. The authors suggest that the contrasting pattern of observed negative social outcomes (reduced sociability and increased aggression) combined with improved social memory as well as increased active coping (in the forced swim test) indicates that early life postnatal stress can cause animals to be become resilient and more capable of coping with stressful situations in later stages of life.

The objective of the study of Varlinskaya et al. (2018) was to systematically test effects of the selective kappa opioid receptor agonist U-62066 on various aspects of social behaviour across ontogeny in rats with a prior history of repeated exposure to restraint. This was done because the dynorphin/kappa opioid receptor system has been identified as a primary target of stress. Social play was assessed as juveniles, adolescents and in adulthood. After stress exposure, animals were treated with the kappa receptor agonist. There was an age-related decline in the amount of social play, juveniles played most followed by adolescent and adult rats. The kappa receptor agonist dose-dependently reduced social play behaviour in both stressed and non-stressed animals regardless of age of testing. In juveniles, vehicle-treated stressed animals played more compared to their non-stressed conspecifics, but this effect disappeared at the later timepoints. Repeated restraint stress in adolescent and adult rats resulted in a decreased baseline level of social investigation and a lower social preference. Furthermore, restraint stress made animals slightly less sensitive to the suppressant actions of the kappa receptor agonist with the most apparent effects in adult social preference. These results indicate age dependent effects of stress on the dynorphin/kappa opioid receptor system in modulating social (play) behaviours.

Papilloud et al. (2018) investigated whether individual differences in stress-induced play fighting will lead to increased adult aggression. To address this question, a rat model of peripubertal stress (PPS) was used which leads to reduced sociability and increased aggression in adulthood. PPS was found to result in enhanced social play in adolescence. By giving animals scores on several play parameters, individuals with high and low levels of play were identified. In adulthood, animals were subjected to a social preference test, a resident-intruder test and a forced swim test. In addition, corticosterone levels, CB1 cannabinoid receptor gene expression in the basolateral amygdala and nucleus accumbens and mitochondrial respiration was investigated in these rats. At the start of the PPS procedure, both high and low playing rats had similar corticosterone levels whereas two days later high players showed lower corticosterone levels compared to low players. PPS animals displayed less social interest in the social preference test and showed increased attacks in the resident intruder test compared to control animals. High players attacked the intruder more abnormally (i.e. no warning signals or at vulnerable body parts). In the forced swim test, PPS animals spent more time floating compared to control animals, suggesting that PPS leads to depressive-like symptoms. PPS did not affect CB1 expression after social play in the basolateral amygdala or nucleus accumbens core but was increased in the nucleus accumbens shell, especially in low players. PPS animals, and especially high players, also showed an increase in mitochondrial function in the amygdala after the social play test. Thus, adolescence may be a critical period in which aberrant social play induced by PPS is linked to adult aggression by altering brain energy metabolism in the amygdala.

These studies suggest that (early) postnatal or prepubertal stress leads to an increase in social play behaviour (see Table 2). The relationship between stress and social play behaviour is still ambiguous, with the starting point, duration and severity of the stressor affecting social play behaviour differently. These effects can be explained by the "rebound effect" where after short, mild to moderate stressors young rats play more as a coping mechanism (Hole, 1991; Thor and Holloway, 1984b; Von Frijtag et al., 2002), whereas more severe stress suppresses social play (Siviy et al., 2006; Siviy and Harrison, 2008).

4.6. Environmental pollutant exposure in the perinatal period

Perinatal exposure to glyphosate-based herbicide (GBH), the most used organophosphate pesticide in the world, on maternal, pup and juvenile social and cognitive behaviour was investigated by de Oliveira et al. (2022). Exposure to glyphosates has been associated with a higher prevalence of ASD (Von Ehrenstein et al., 2019). The neuro-behavioural assessment of the offspring showed reduced time spent on social play behaviour, impaired homing behaviour, and increased stereotypic behaviour in both males and females. Object recognition was impaired only in the male offspring of GBH-exposed mothers. Oxidative stress markers in the prefrontal cortex and hippocampus were altered due to maternal exposure to GBH. The data thus show altered oxidative stress markers and an ASD-like phenotype due to maternal exposure to GBH.

Exposure to airborne particulate matter has been associated with ASD, cognitive delay, depression, anxiety and neurodegenerative diseases possibly via neuroinflammation and oxidative stress. In the study of Nephew et al. (2020) animals were exposed throughout gestation and lactation to traffic related air pollution at exposure-levels that were epidemiologically observed in metropolitan areas. Rats in the exposure group displayed less social play (dorsal contacts and wrestling) and allogrooming. Next to reduced social behaviour, ex-vivo DTI-MRI showed disrupted neural integrity in the hippocampus and anterior cingulate cortex of exposed juvenile animals.

Although different types of stimuli, exposure to the environmental endocrine disruptor, bisphenol A and a high-fat diet are prevalent in industrialized societies. In the study of Wise et al., (2019), the effect of the combination of these two environmental exposures on maternal behaviour, social behaviour of the offspring and gene expression and cytokine levels in the medial prefrontal cortex was investigated. Pregnant dams consumed 3 doses of bisphenol A and were fed either a control or high-fat diet from gestational through parturition. Social play was reduced and passive social contact increased by the highest bisphenol A dose on day 3 and 4 compared to day 1 and 2 of observation. Furthermore, the diet did not affect social play behaviour. Bisphenol A did not affect maternal behaviour whereas a high-fat diet resulted in more time spent nursing the pups. Perinatal bisphenol A and high-fat diet did not affect anxiety in the elevated plus maze or social recognition in the 3-chamber task. In males several pro-inflammatory cytokines were increased by the highest dose of bisphenol A in the medial prefrontal cortex, whereas females remained unaffected. From all the genes assessed, estrogen receptor 1 expression was increased in females on PND10 and PND90 after bisphenol A exposure. In this study, bisphenol A and high-fat diet affected behaviour and gene expression but they did not have a synergistic effect on behaviour or inflammation markers in the brain.

Kougias et al. (2018) used a rat model of human prenatal phthalate (a plasticizer) exposure to investigate the potential interactive effects of an environmentally relevant mixture of phthalates and a maternal high-fat diet. From gestation through PND10, dams consumed the mixture of phthalates and a diet. The authors found that in males, perinatal exposure to the mixture of phthalates decreased body weight and dose-dependently reduced social play behaviour. Females actively avoided social interaction in a dose- and diet-dependent manner. There was also a tendency for increased oxidative stress markers on PND10 within the medial prefrontal cortex of males exposed to a high dose of phthalates combined with high-fat diet. The authors concluded that phthalates and a maternal high-fat diet rarely interact but that perinatal exposure to phthalates has a lasting impact on social behaviours in both males and females.

These studies show that exposure to commonly used chemicals in the environment affect brain development and reduce social play behaviour (see Table 2).

4.7. Other pre-, peri- or postnatal treatments affecting social play

Perinatal asphyxia is also thought to be involved in the development of ASD, ADHD and schizophrenia. In the study of Vázquez-Borsetti et al. (2019) male pups were subjected to asphyxia at birth either by immersing them for 19 min in warm or cold water-baths. Afterwards one group was exposed to hypothermic shock whereas the rest was exposed to heat mats. This resulted in five groups: 1. Vaginal delivery control, 2. Caesarean control, 3. Postnatal asphyxia normal temperature, 4. Postnatal asphyxia hypothermia and 5. Postnatal asphyxia with postnatal hypothermic shock. Social behaviour, including social play and behaviour in the open field was assessed. Exposure of male rats to postnatal asphyxia suppressed play initiations that could be prevented by postnatal hypothermic shock but not by hypothermia. Locomotion was unaffected in all groups. Investigating the brain showed that the amount of GABAergic neurons was unaltered due to the treatments in both the medial prefrontal cortex and the anterior insular cortex but layer specific analysis showed differences in the distribution of subtypes of GABAergic neurons (expressing reelin or calbindin). In layer VI of the medial prefrontal cortex, a reduction in the amount of reelin-GABAergic neurons due to asphyxia was found that could not be prevented hypothermic shock. Asphyxia resulted in an increase in the number of reelin-secreting GABAergic neurons in layers II and III but resulted in a reduction of calbindin-GABAergic neurons in layer II of the anterior insular cortex. Both effects could be prevented by the hypothermic shock. These results add to the literature on the importance of proper development of cortical regions and excitatory to inhibitory balance in modulating social play behaviour.

de Novais et al. (2021) assessed the effect of an overweight mother on brain and behavioural development of the offspring because maternal obesity is associated with neurodevelopmental disorders in the offspring. A small litter size induces maternal overweight, therefore litter-size was included as a factor and maternal as well as pup behaviour was assessed in the second generation. Total time spent on social play was reduced in both sexes in second generation offspring with an obese mother. The other behavioural tests of social interaction, communication, locomotion and repetitive behaviour point to an ASD-like phenotype, especially in male offspring. Synaptophysin (a marker for synaptic plasticity and integrity) levels were affected in the hippocampus (reduced in males and increased in females) and prefrontal cortex (reduced in females). These results show transgenerational effects of environmental manipulations on social play behaviour.

Concussions or mild traumatic brain injuries (mTBI) resulting from falls and impacts are not uncommon when children play. While most children are not affected by this experience, some children experience pervasive cognitive and/or motor impairments after mild traumatic brain injuries. Mychsiuk et al., (2014) found that mTBI and sex influenced how rats played. Females in a pair containing at least one mTBI animal show less play initiations which was not the case for males. Microstructural play analysis revealed that male control rats were significantly less likely to initiate play with an mTBI animal. Similarly, they found that female control rats initiated high levels of play with other control animals but were less likely to initiate play with mTBI animals. In addition, they found that both male and female mTBI rats will initiate play with control animals but females will not do so with other mTBI females. Dyck and Ivanco (2018) investigated how mild traumatic brain injuries to the motor cortex affects social play behaviour and how brain-derived neurotrophic factor (BDNF) levels are affected. BDNF is a protein known to be involved in the growth and survival of neurons in response to injury. In contrast to Mychsiuk et al., (2014), no differences in social play behaviour was observed between the groups in both sexes. In addition, BDNF levels in the injured areas (left or right motor cortex) were higher in animals exposed to mild traumatic brain injuries and no difference was found in the medial prefrontal cortex. Together these data show that mTBI can affect social behaviour and brain development.

The studies described show a variety of environmental physical or psychological and even transgenerational insults and their effect on brain and behaviour, including social play (see Table 2). This once more highlights the sensitivity of this behaviour and its potential role as an early marker for adversity in adulthood.

4.8. Summary

The studies described in this section show a variety of environmental physical or psychological and even transgenerational insults that directly or indirectly affect social play behaviour (see Table 2). Social play is predominantly displayed in the juvenile period, when the development of the social brain and social, emotional and cognitive development is ongoing. These models improve our understanding of the neurobiology of social play by either directly or indirectly acting on neural systems known to be involved in social play. The VPA models show that excitatory to inhibitory balance in the brain, and that endocannabinoid, serotonin and dopamine tone in play-associated brain regions are important for normal expression of social play. The ASD risk gene studies highlight the importance of proper synaptic structure and plasticity whereas the MIA studies demonstrate altered activity of play-associated brain regions likely due to inflammatory processes. The adversity studies show the vulnerability of the brain to stress in early development and that play levels correlate with corticosterone and CB1 receptor levels affecting brain metabolism. The environmental pollution studies revealed an association between oxidative stress markers, pro-inflammatory cytokines, disrupted neural integrity and a reduction in play whereas stressors also affect the integrity of the mPFC, important for proper execution of social play and interpretation of social signals. On the other hand, by including this easy to measure behaviour, this offers insight into the effects of early manipulations at an important developmental timepoint as an potential early marker for social, emotional and cognitive impairments in adulthood, thereby expanding the "operational range" of animal models. When social play is affected this might also offer specific timepoints for treatment development to reverse or alleviate the impact on adult social behaviour.

5. Discussion

In this review we aim to provide an updated overview of the literature on social play behaviour since our previous reviews (Vanderschuren et al., 2016; Trezza et al., 2019) but this time from two perspectives. The first section discussed the neurotransmitter systems and the brain regions involved in the expression of and motivation to play. Opioids, cannabinoids, dopamine, noradrenaline, serotonin and vasopressin modulate social play behaviour. What is rapidly developing over the past few years is brain region specific manipulations, allowing us create a better picture of the neural circuitry underlying the rewarding and motivational aspects of this behaviour. With this clearer picture of the neurocircuitry of social play, we can compare this social natural reward with the neurocircuitry of other natural rewards such as food, to other social rewards such as sex and artificial rewards such as drugs of abuse. In this way it is possible to highlight differences, similarities and unique aspects defining the neural mechanisms of social play behaviour. With the emergence of more fine-grained techniques to target, manipulate and visualize neuronal sub-populations, receptors and signaling molecules, this picture becomes increasingly more complete as well as detailed.

In the second part a summary is given of how social play is increasingly being incorporated in animal models of neurodevelopmental disorders and (transgenerational and/or early) environmental insults, thereby providing insight into the neural mechanisms underlying this behaviour in addition to broadening the range of social behaviour to be assessed at important developmental timepoints. Furthermore, by including the juvenile period with an easy to quantify behaviour this expands the time-window for the development of interventions.

A possible route for intervention development could arise from the neuromodulators described in the first section. Direct successful examples of this are the studies of Servadio et al. (2016) and Melancia et al. (2018), in which VPA-induced reductions in social play behaviour as well as other core symptoms of ASD (anxiety, impaired vocal communication and stereotypic behaviour) were rescued by treatment with the anandamide hydrolysis inhibitor URB597, highlighting the importance of the endocannabinoid system in social play and ASD-like symptomatology. Also in the VPA model, Román et al. (2021b) showed that the dopamine D3-preferring D3/D2 receptor partial agonist cariprazine could rescue the decrease in social play expression. Other drugs for potential treatments are mu-opioid receptor agonists to enhance play expression or increasing dopamine levels to enhance the motivation to play (see Table 1). However, the disadvantages of these drugs are their abuse potential and their effect on other types of behaviour because of similarities in underlying neurocircuitry. Other interventions that could be explored are behavioural interventions as early as maternal behaviour (e.g. raising an affected pup with a non-affected mother), housing with a playful strain and environmental manipulation such as environmental enrichment. These are discussed below (see 'Broadening the field').

5.1. A note on how social play is investigated: differences and similarities in methodology

With the development of detailed staining and visualization techniques, specific neurobiological techniques such as in vivo electrophysiological recordings, opto- and chemogenetics, fiber photometry in combination with specific genetic animals models as well as the increased use of artificial intelligence, scientists are now able to form and test very specific questions about the neurocircuitry of social play behaviour. Furthermore, it challenges researchers to adjust these techniques for use in young playing rats. Since the emergence of reviews on social play in the literature, methodology has been a subject of discussion. In a recent review by Pellis et al. (2023), several set-ups to study social play are discussed depending on the research question of interest. Most pharmacological manipulations have been carried out in a dyadic social interactions set-up, which seems quite straightforward. However, taking into account Table 1, one can appreciate the diverse possibilities of setting up a dyadic social encounter.

It starts with the selection of the strain of animals, which is known to affect the "baseline" amount of play expressed (Siviy, 2020; Manduca et al., 2014; Himmler et al., 2013, 2014). From the 139 experiments displayed in Table 1, 97 are carried out in Wistar, 22 in Sprague-Dawley, 11 in non-specified albino strains and 9 in Long Evans rats. Next is the age during the peak play period at which juveniles are assessed. Thor and Holloway, 1984c reported that the peak for social play behaviour is around PND35–36, whereas the experimental data displayed in the table ranges from as early as PND21 up to PND50.

It has been demonstrated that the amount of social isolation/play deprivation before a dyadic test modulates the motivation to play (Niesink and van Ree, 1989; Achterberg et al., 2016; Vanderschuren et al., 1995; 2008). Of the studies reviewed, the amount of social isolation varies between no isolation at all to continuous social isolation during the juvenile period, with the most used isolation period being between 2 and 4 hrs. Also the observation time differs from 5 to 60 min with continuous observations to sampling every 1 or 5 min, of which 10–15 min continuous observation is used most often. The one experimental parameter that varies the most is the characteristics of the dyad in the testing environment. This ranges from same-sex, similar background/treatment, unfamiliar, weight-matched individuals to a partner that is naïve, with every possible variation between these options being reported as well. Lastly, the way social play behaviour is reported in the literature differs between labs, with frequencies, durations and percentages of time spent on (combinations of) behaviours used most often.

When listing these factors in the studies where social play used as a parameter (Table 2), these same differences in the testing of social play are observed.

Importantly, we did not include handling and habituation procedures, whether the animals are tested in the home-cage or a test-box, characteristics of the test-box itself, day-night cycle of the animals, time of testing during the day, the lighting conditions during testing or even the ethogram used in the table but there are considerable differences in these procedures as well. On the positive side, general effects of certain drugs have been observed despite these differences, such as for morphine, naloxone, methylphenidate and flupenthixol (see Table 1). However, with the increasingly specific studies into the neurocircuitry of social play it becomes more difficult to directly compare studies from different labs. When comparing studies from different labs this should be taken into account, with the first step being as complete as possible in the method section of articles, in order to be able to gauge whether discrepancies between studies can be traced back to methodological differences.

5.2. Broadening the field

A great part of the literature on social play behaviour describes dyadic social play of which the underlying neurobiological mechanisms are becoming more and more clear. Next to that, analysing this behaviour in longitudinal neurodevelopmental studies allows for potential early detection of social behaviour problems in animal models of developmental and/or psychiatric disorders or effects of environmental manipulation. This increase in knowledge also results in interesting new avenues of which a few are highlighted. The first two new ways of investigating social play go beyond the dyadic social encounter and focus on 1. individual differences in social play, taking into account how a juvenile rat in a dyadic social situation contributes to the total amount of play based on its internal preferred level of play and that of its partner; 2. studying social play behaviour at a group level, which provides information on how animals select certain partners to play with when given the choice. Both these approaches may provide new information on the underlying neurobiology. The other three new avenues are potential behavioural interventions that could be used either alone or in combination with pharmacotherapy to alleviate decreases in social play behaviour displayed in several animal models for disorders of effects of stress (Table 2), i.e. 3. social play used as "therapy", 4. the influence of housing conditions on social play behaviour and 5. interspecies play/simulation of social play.

Individual differences in play: Some rats appear to be consistently more playful than others (Pellis and McKenna, 1992; Lampe et al., 2019), with individuals initiating more play in a dyadic test also initiating more play in the home cage (Lampe et al., 2017; Melotti et al., 2014). Lesscher et al. (2021) attempted to capture these individual differences by assessing the amount of play during two test-days. The authors found significant differences in play between individuals, named low and high playing rats. When these groups when subsequently exposed to alcohol in adolescence, high players drank more than low players. These differences in alcohol intake persisted into adulthood. High players were shown to have more behavioural control over alcohol seeking compared to low players in a conditioned suppression test. This suggests that individual differences in social play have consequences for adult behavioural and cognitive performance. In Pellis et al. (2022) individual differences in social play (in this case assessed with three test-days) were correlated to USV emission to assess whether communication between high and low players differs during play. Here, the authors found that high and low players use different attack and defence postures (see Pellis et al., 2022 for microstructure of social play); high players not only played more but they also use a form of play that requires close-quarter contact. Interestingly, although high and low players emitted the same amount of calls, they show a different pattern of how play behaviour is associated with certain USVs. The low players show stronger associations between certain play behaviours and USV type, which the authors interpreted as the low players using the calls more strategically to coordinate their social play (Pellis et al., 2022). These articles indicate that the field of social play behaviour is broadening by combining approaches to assess social play as well as including ultrasonic communication, taking into account behaviour at the individual level and how this affects behaviour in relevant challenging situations.

Social play assessment in groups: In line with the broadening of the study of social play behaviour, an increased interest is shown towards social play in group settings. From a translational perspective this trend is more naturalistic, however, the challenge with these experiments is the increased amount of time for behavioural observations. Answering questions about social play in groups is aided by artificial intelligence-based methods that track animals and interpret certain types of behaviour, although for complex behaviour like social play behaviour this remains challenging. Lampe et al. (2019) used triads to assess how animals with a certain play level (high, intermediate or low) would play with animals having similar or dissimilar play levels. They found that similarity in play levels promotes engaging in social play behaviour. To investigate the effect of prenatal alcohol exposure on social behaviour, Holman and colleagues (2019) took the triad approach to assess how alcohol exposed-rats interact socially. Their hypothesis was that the social environment may subserve prenatal alcohol exposure-related social behaviour deficits in more complex social contexts. The authors showed that when a triad consisted of one alcohol-exposed and two control rats, the control animals spent significantly less time playing with the alcohol-exposed cage-mate. These data support the idea that prenatal alcohol exposure affects social competence when faced with "normal" play partners. In Pellis et al. (2022) groups of 6 animals were followed and social network analysis was used to assess play preferences, revealing that individual rats indeed have play-mate preferences while still playing with all members of the group. Together with the work on individual differences in social play specific questions about brain- and behavioural development in more complex social settings can be assessed.

Social play as therapy: To answer questions about the function of social play, research has mainly focused on social isolation or play deprivation, leading to valuable insights (for reviews see Pellis et al., 2023; Vanderschuren and Trezza, 2014). From another perspective, it is interesting to investigate whether supplementing the opportunity to play could prevent or alleviate deficits due to early life adversity or structural brain alteration. Panksepp and colleagues proposed this approach already in 2003, where they used novel similar-treated animals as play therapy for rats with unilateral neonatal frontal lobe damage. This frontal lobe damage resulted in increased play levels which could be returned to control levels by nine days of play therapy, comprising 2 daily play sessions of 30 min (Panksepp et al., 2003). Nwachukwu et al. (2021), used play therapy to investigate how to counteract or prevent the negative effects of repeated mild stress. During four weeks of unpredictable predator-related stressors, juvenile rats received the plant-based antidepressant icariin (Wei et al., 2016) alone or in combination with daily 5 min play sessions with an animal from another home-cage. After the four weeks of stress and treatment, stress measures were assessed before and after the forced swimming test. The stress measures of metabolized corticosterone, DHEA and testosterone or behaviour in the swim test were not affected by the opportunity to play. These findings might be due to the short amount of time rats had the opportunity to play or that the forced swim test is not sensitive to the effects of social play. Even so, on balance the available data suggest that supplementing play may lead to non-pharmacological therapies to improve behaviour and brain function.

Housing conditions: environmental enrichment: Cutuli et al. (2019) investigated the growing evidence that parental environmental enrichment, even occurring in the pre-reproductive phase, affects behavioural and neural development of the offspring. Specifically, they examined the

effects of pre-reproductive enrichment of dams on the play performances of their male and female adolescent offspring. In addition, the effects of pre-reproductive enrichment on maternal behaviour and male intruder aggression was examined, as well as the number of oxytocinergic neurons of the paraventricular and supraoptic nucleus of the hypothalamus in both dams and offspring. In juvenile animals from standard-housed mothers males played more than females, while no sex difference was observed in animals born to enriched housed mothers. Compared to offspring from standard-housed mothers, enriched housed males pounced less, while the opposite effect was found for females. Female rats from environmentally enriched mothers showed increased numbers of oxytocin cells in the paraventricular and supraoptic nucleus in adulthood but not adolescence, whereas in males the number of oxytocin cells was increased in the supraoptic nucleus in adolescence. These data, together with the data from Kentrop et al. (2018) show that housing condition is a factor influencing the amount rats play in a sex-dependent way.

Interspecies social play: next to social play with conspecifics it is also possible to play socially with other species. This approach can give insight into what aspects are important for social play behaviour, especially when body size and communication differs substantially. Heterospecific play, an experimenter 'tickling' rats, is thought to simulate conspecific play, and has been used to assess and improve welfare and to study the neurobiology of positive affect and communication (LaFollette et al., 2017; Bombail et al., 2021; Wöhr et al., 2009). Burke et al. (2022) compared play behaviour-USV associations between playing with a conspecific and being tickled by an experimenter. They showed that USV emission was prevalent in both types of play but that the different types of USVs are used in the two contexts was considerably different. The authors concluded that both types of play are positive experiences but that tickling is not the equivalent of conspecific play. How the brain is influenced by playing with humans is studied by the Brecht lab. Reinhold et al. (2019) developed a hide-and-seek game for rats with their human experimenters where rats take both the roles of hiders and seekers. Like social play behaviour with conspecifics this type of play was found to engage the medial prefrontal cortex. Concha-Miranda et al. (2020) expanded on this research by showing that medial prefrontal cortical neuronal population activity is higher during play performance compared to observing play.

Social play behaviour is increasingly incorporated in longitudinal studies of genetic, early life events and environmental factors. The study of social play behaviour in rats itself is broadening from observing behaviour in dyads, which is important in answering questions about the involvement of brain systems to the individual rats contribution to dyadic or group play. Also relevant measures such as ultrasonic communication is adding to the picture what is needed for a successful social play interaction. Next to that, new frontiers such as play supplementation and interspecies play are being explored and these create an increasingly complex but clearer picture about this behaviour which ultimately aides in understanding animal social behaviour.

5.3. The future: outstanding questions

In this section, we would like to point out several outstanding questions which will further expand and challenge the field of play research, and in the end, deepen our knowledge of this fascinating behaviour. The fact that the lion's share of work into the neural mechanisms of social play behaviour to date has been performed in male juvenile rats indicates three important areas in which our knowledge needs to be increased.

The first question that needs to be addressed is whether play behaviour comes about through different mechanisms in male and female animals, in which case it is probably most feasible to extend the existing work in rats. Early studies suggested that sex differences in social play behaviour in rats are quantitative, in the sense that male rats play more than female rats (Meaney and Stewart, 1981; Poole and Fish 1976). However, subsequent studies did not consistently find these sex differences. Rather, detailed analysis by Pellis and colleagues revealed qualitative differences in the structure of social play behaviour in male vs female rats (Pellis, 2002; Pellis et al., 1997). Importantly, there is emerging evidence for differences in the neural mechanisms of social play in male and female rats, for example in the involvement of microglia shaping these sex differences in the brain (VanRyzin et al., 2019) via cannabinoid (Argue et al., 2017) and dopaminergic (Kopec et al., 2018) mechanisms. Next to that, social play is modulated differently in males and females by septal dopamine and vasopressin (Bredewold et al., 2018; Veenema et al., 2013). In addition, work using prenatal VPA treatment to model autism-like behaviour has also revealed distinct effects on social play in males vs females (Gzielo et al., 2020; Melancia et al., 2018). These findings emphasize the need to include both male and female animals in future studies on the neurobiology of social play behaviour (see e.g. Marquardt et al., 2023).

The second question relates to whether our knowledge on the neurobiology of social play behaviour in rats is comparable to other (non-rodent) species. As far as our knowledge goes, there is a modest literature on the neural mechanisms of social play in hamsters (e.g. (Burleson et al., 2016; Cheng and Delville, 2010; Cheng et al., 2008; Murphy et al., 1981), and a number of studies in non-rodent species, including dogs (Romero et al., 2015) and non-human primates (Guard et al., 2002; Madrid et al., 2018; Wellman et al., 2016; Zhou et al., 2019). This question is important for both fundamental and translational reasons. From a fundamental point of view, similarities as well as differences in the neural mechanisms of social play between rats and other species can inform about the structure and function of this behaviour between species. Structural differences in social play have been described among rodent species (Pellis and Pellis, 1998), and given the notion that social play is thought to be important for the appropriate development of an adult behavioural repertoire, it is likely that the structure and function of social play also differs between prey and predatory species (Graham and Burghardt, 2010). From a translational perspective, there is an urgent need for (pharmacological) therapies that can alleviate the social deficits in psychiatric disorders such as ASD and schizophrenia, some of which – especially in ASD – are present from a young age onwards (Jordan, 2003; Møller and Husby, 2000). Therefore, translating our expanding knowledge on the neural underpinnings of social play in rats may inform the development of (pharmaco)therapies for social deficits in humans.

Last, we would like to address age-related matters. Social play behaviour is most abundant in young – juvenile and early adolescent – animals, and it wanes in intensity and changes in structure during the transition into adulthood (see e.g. Baenninger, 1967; Meaney and Stewart, 1981; Panksepp, 1981; Pellis and Pellis, 1990 for studies in rats). However, social play is far from absent in adult animals and humans (for a recent review see Palagi, 2023). About this point, we think that the -quote by George Bernard Shaw ('We don't stop playing because we grow old; we grow old because we stop playing') speaks volumes. This raises two further questions. First, as the abundance and structure of social play changes from weaning to adulthood, do the underlying neural mechanisms change too? It is plausible to think that the answer is yes, but as far as our knowledge goes, there are no studies that have explicitly addressed this question. Clearly, information about the – changing – neurobiology of social play during development is likely to inform about its relevance and function as well. Second, does the function of social play change with age? It is well accepted that social play functions to facilitate the development of social, emotional, cognitive as well as physical skills, to equip the adult animals with an adaptive and flexible behavioural repertoire (Bateson, 2015; Graham and Burghardt, 2010; Panksepp et al., 1984; Pellis and Pellis, 2017; Špinka et al., 2001; Vanderschuren and Trezza, 2014), whereas in the short term, by virtue of its rewarding properties, it contributes to emotional wellbeing (Held and Špinka, 2011; Trezza et al., 2011a; Vanderschuren, 2010). To a certain extent, social play in adults could

subserve both functions, but perhaps to a different degree. Being pleasurable likely remains an important property of social play in adults, as having fun with others is probably one of the most important aspects of social interaction. More generally, it has been proposed that social play in adults may function to assess and manipulate social relationships (Pellis and Pellis, 2009; Palagi, 2023). A developmental function of adult social play then, likely refers to the property of the adult brain to stay plastic throughout life, albeit to a lesser extent than during youth. Stated otherwise, adult social play may subserve an ongoing function to maintain and extend the behavioural repertoire as a function of adult social experience – which has also been proposed to be a function of adult object play in primates (Nahallage et al., 2016).

6. Conclusion

The present review summarizes recent progress in our understanding of the neural underpinnings of social play behaviour. As we hope that the reader will appreciate, the first two decades of the 21st century have seen a substantial surge in social play-related studies. We hope that this development will continue in the years and decades ahead of us, not least since we think that social play behaviour is an important driving force of brain development in the young, and of mental health in the adult, especially as they age.

Data Availability

No data was used for the research described in the article.

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