4-15-2016

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Siviy, Stephen M. "A brain motivated to play: Insights into the neurobiology of playfulness." Behaviour 153 (2016): 819-844.

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Keywords
amygdala, cortex, genetics, motivation, play, postnatal, rat, striatum

Disciplines
Cognitive Psychology

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Play is an important part of normal childhood development and is seen in varied forms among many mammals. While not indispensable to normal development, playful social experiences as juveniles may provide an opportunity to develop flexible behavioral strategies when novel and uncertain situations arise as an adult. To understand the neurobiological mechanisms responsible for play and how the functions of play may relate to these neural substrates, the rat has become the model of choice. Play in the rat is easily quantified, tightly regulated, and can be modulated by genetic factors and postnatal experiences. Brain areas most likely to be involved in the modulation of play include regions within the prefrontal cortex, dorsal and ventral striatum, some regions of the amygdala, and habenula. This paper discusses what we currently know about the neurobiological substrates of play and how this can help illuminate functional questions about the putative benefits of play.

Introduction

To be playful is a state where many adult humans would like to be and where most children should be. Indeed, play is almost synonymous with a happy childhood and is a major component of the behavioral repertoire of many mammalian species. Conversely, the lack of play in an otherwise healthy child can be cause for concern as almost all psychiatric disorders include some type of social dysfunction as a core symptom. In the child this could easily manifest primarily as dysfunctional social play. This makes the presence or absence of play a useful diagnostic indicator when assessing the emotional well-being of a child and one of several end-points when assessing treatment options. But what exactly does it mean to be playful? More importantly within the context of the present paper, how can we characterize play in terms of those brain mechanisms responsible for modulating this state and can understanding these brain mechanisms glean insight into the overall function of play?

Play can take many forms (Burghardt, 2005). For some species, such as humans, play can be incredibly diverse and range from solitary imaginative play to highly energetic rough-and-tumble social play. For other species, such as the laboratory rat, play is to the best of our knowledge limited to social rough-and-tumble interactions. Since this paper will be focusing on work done with the laboratory rat, the use of the term "play" will for the most part be limited to social rough-and-tumble play. With this caveat in mind, the young of many mammalian species engage in some type of social behavior that can be easily identified as playful. This suggests that the neural circuitry responsible for modulating play may have evolved early in mammalian evolution and is likely to be fairly well conserved across those mammalian species that engage in play. While the exact structure of the behavior may differ between those mammalian species that play it is the premise of this paper that the core neural circuitry that motivates an animal to engage in playful social interactions is shared among these species. Given that some type of play has also been reported in birds, reptiles, and even amphibians (Burghardt, 2005), it is also possible that the evolution of basic relevant circuitry even pre-dated mammals. Whether these circuits are homologous to those responsible for mammalian play is a matter of speculation at this point. However, it does suggest the possibility that play as a stable behavioral phenotype, and the underlying

circuitry, may have appeared sporadically throughout evolution but may have become more firmly established in mammals.

Play is also important for the overall development of the animal. For example, when deprived of the opportunity to play as juveniles and then assessed as adults, rats are impaired socially, emotionally, and cognitively (Baarendse et al., 2013; Van den Berg et al., 1999; Vanderschuren & Trezza, 2014; Von Frijtag et al., 2002). This would suggest that the young mammalian brain is programmed and motivated to engage in playful behaviors, with adverse consequences resulting when opportunities for play are thwarted.

The rat as an optimal animal model to study the neurobiology of play

As has been the case when studying many other motivated behaviors, the standard laboratory rat has been an optimal model system for studying the neurobiology of mammalian playfulness. Unlike its cousin the mouse, another excellent model system for studying mammalian behavior, rats are extremely playful prior to puberty. Rats begin to engage in playful interactions prior to weaning and this continues throughout the juvenile period, peaking at around 35 days of age, and then steadily decreasing as the animals reach puberty (Panksepp, 1981). Play in the rat primarily takes the form of "rough-and-tumble" activity; rats will vigorously chase each other, pounce on each other's dorsal surface, nuzzle and nip at the nape, and pin each other (Panksepp et al., 1984; Pellis & Pellis, 2009; Siviy & Panksepp, 2011; Vanderschuren et al., 1997; Vanderschuren & Trezza, 2014). Although dominance-related asymmetry can occur between two rats (Pellis et al., 1993b; Smith et al., 1996), both partners of a play dyad tend to get a fair share of pouncing and pinning during a bout of play. Play is also distinct from other categories of social behavior in the adult rat, such as aggression and sex, and while play may borrow elements from these other behaviors these are combined in ways that clearly distinguishes the overall activity from the adult counterparts (Pellis & Pellis, 1991).

Play is quite sensitive to variations in motivational state and is under fairly tight regulatory control. For example, the amount of play observed during a discrete observation period (e.g., 5 – 15 minutes) can be readily titrated by housing rats in isolation for varied amounts of time (Niesink & Van

Ree, 1989; Panksepp & Beatty, 1980; Siviy et al., 1997); a rat that has been isolated for 4 hours will play more than a rat that has not been isolated, and a rat that has been isolated for 24 hours will play more than a rat isolated for 4 hours. In addition to telling us something about the motivational dynamics associated with play, this level of regulatory control is also very advantageous when designing experiments to look at the neural control of play. For example, most pharmacological manipulations have a limited window of bioavailability so timing between injection and testing can be optimized to insure that any behavioral effect is likely to be seen. Different components associated with social interactions between juvenile rats may also be variably sensitive to prior social isolation. For example, Niesink & Van Ree (1989) report that only 3.5 hours of isolation was necessary to yield a maximal increase in social grooming while at least 24 hours of isolation was needed to reach a maximal increase in pinning.

Several complementary paradigms have been utilized to directly address the affective and motivational side of play in the rat. When tested in a conditioned place preference paradigm, rats will prefer to return to a unique environment where play was experienced over an environment not associated with play (Calcagnetti & Schechter, 1992; Trezza et al., 2009). This demonstrates that play is indeed a pleasurable experience to the rat. Rats will also readily perform operant responses when an opportunity to play is the reward. Early studies showed that juvenile rats would readily learn to navigate a T-maze when one arm was baited with another juvenile rat ready to play (Humphreys & Einon, 1981; Normansell & Panksepp, 1990). A novel paradigm where rats must perform an operant lever-pressing task in order to obtain a brief opportunity to play has recently been developed (Achterberg et al., 2016; Trezza et al., 2011a; van Kerkhof, 2013) and this approach should allow for a more thorough assessment of factors involved in the motivation to play.

Rats exhibit high frequency (50 – 55 kHz) ultrasonic vocalizations (USVs) when playing and when anticipating play, both of which have been interpreted as markers of a positive affective state (Burgdorf et al., 2008; Knutson et al., 1998, 2002). These USVs may also serve a communicative function as the playback of 50 kHz vocalizations will readily elicit approach (Seffer et al., 2014; Willadsen et al., 2014). When tracked over the course of a play bout, USVs are more likely to occur

shortly before playful contact is made and this would be consistent with rats using USVs to communicate playful intention (Himmler et al., 2014). However, two studies looking at play in devocalized rats suggest that caution should be used before concluding that 50 kHz USVs serve as play signals (Kisko et al., 2015a, 2015b). For example, if vocalizations are used to signal playful intent and help maintain the vigor of a play bout, then devocalized rats should be expected to be less playful. Indeed, devocalized rats that are paired together will direct fewer contacts to the nape than pairs of sham-treated rats which is consistent with the hypothesis that USVs help signal playful intent and maintain playful motivation. However, it was also found in the same study that devocalized pairs were more likely to respond contacts directed to the nape by rotating completely to a supine position (i.e., allowing them to be pinned). Since pinning is thought to help maintain and prolong the playfulness of a social interaction (e.g., Panksepp et al., 1984; Pellis et al., 1997; Vanderschuren & Trezza, 2014) this is the opposite of what would be predicted if USVs enhance play motivation. Furthermore, pairing a devocalized rat with an intact unfamiliar rat had little impact on play. So while the extent to which 50 kHz USVs represent true play signals is unclear, inclusion of this measure as a putative index of underlying affect during play is sure to add additional insight into the various components of play in the rat and the neurobiological substrates associated with them.

The amount of playfulness exhibited by a rat has been shown to be sensitive to both genetic and early postnatal influences. For example, the inbred F344 rat is consistently less playful than other inbred and outbred rats (Siviy et al., 1997, 2003, 2011), as is the Spontaneously Hypertensive Rat when compared to the normotensive Wistar-Kyoto strain and the outbred Sprague-Dawley strain (Ferguson & Cada, 2004). When both play and USVs are examined simultaneously, Sprague-Dawley rats were found to be more playful than Wistar rats and emitted more USVs during playful social interactions (Manduca et al., 2014). However, Sprague-Dawley rats also emitted more USVs than Wistar rats during non-social exploration of the cage and when self-grooming; while morphine increased play in both strains, it did not increase USVs further. These data suggest that genetic influences on playfulness may not map equally onto all aspects of playful interactions.

Rats selectively bred for certain physiological and/or behavioral traits have also been shown to systematically differ in playfulness. For example, rats that have been selectively bred to be susceptible to amygdala kindling play more than those that are resistant to amygdala kindling (Reinhart et al., 2004, 2006). Rats exhibit high-frequency vocalizations when tickled by human experimenters (Burgdorf & Panksepp, 2001) and rats selectively bred for high rates of ticking-induced vocalizations solicit more play than those bred for low rates (Webber et al., 2012). Similarly, infant rats bred for either high or low separation-induced high-frequency vocalizations play less than random control lines when tested as juveniles (Brunelli et al., 2006). Taken together, these data suggest that the amount of playfulness exhibited by a rat can be systematically modulated by genetic variability.

Genes aren't everything, however. There is considerable evidence highlighting the impact that early post-natal experiences may have on play as well. It has been known for some time that early interactions between mother and pup can have lasting consequences on the affective lives of rats as juveniles and adults. For example, rats that receive more licking and grooming from the dam (high-LG) as pups tend to be less fearful (Menard et al., 2004), are more likely to explore a novel environment (Caldji et al., 1998), and have an attenuated startle response (Zhang et al., 2005) when tested as adults and when compared to rats raised by dams that engage in less licking and grooming (low-LG). Rats that experience brief (e.g., 15 minutes) periods of maternal separation during the first 2 weeks of life are also less anxious and less stressed as adults (Boufleur et al., 2013; Madruga et al., 2006; Meerlo et al., 1999; Raineki et al., 2014). With regards to play, it has been reported that male pups of low-LG mothers tend to be more playful when play is assessed in the home cage and when housed together with male pups of high-LG mothers and female pups of both low-LG and high-LG mothers (Parent & Meaney, 2008). These findings corroborate an earlier study showing that male rats receiving less licking and grooming of the genital area play more than those receiving more licking and grooming (Moore & Power, 1992). However, these findings are in contrast to two reports showing that male and female pups that experienced brief (e.g., 1 – 15 minutes) daily periods of separation from the mother play more than those from undisturbed litters (Aguilar et al., 2009; Siviy & Harrison, 2008). This is particularly intriguing

since this procedure of brief daily maternal separation, also known as “handling”, has been reported to increase the amount of licking and grooming by the mother towards her litter (Liu et al., 1997) and, as mentioned above, leads to the same effects on measures of stress and anxiety as does being raised by a high-LG mother.

There are several methodological differences between these two experimental approaches that may help explain these disparate results and may also shed some light on how early experiences can impact playfulness. First, those studies described above that looked at natural variations of maternal behavior assessed play of rats in their home cages using a focal observation method while the studies assessing the effects of “handling” assessed play in a discrete paired-encounter procedure after a period of social isolation. It is possible that the amount of licking and grooming received by a pup during the first 2 weeks of life may be having more of an impact on brain mechanisms associated with motivational factors and may not be as easily detectable when animals are being observed in the home cage. Consistent with this interpretation is a recent study looking at play as a function of within-litter variation in maternal care (van Hasselt et al., 2012) where a significant positive correlation was found between the amount of licking and grooming received by male rats during the first week after birth and frequency of pinning, pouncing, and latency to initial social exploration. In other words, male rats receiving more licking and grooming as a newborn were more playful and more socially curious as juveniles. Interestingly, variability in play among females was unrelated to amount of licking and grooming received by the dam as pups which is consistent with earlier studies (Moore & Power, 1992; Parent & Meaney, 2008). It is also perhaps significant that play was assessed during a discrete 15 minute observation period and after 3 hours of isolation. Unfortunately, the two “handling” studies mentioned above did not quantify maternal behavior, leaving open the possibility that the manipulation used in these studies did not increase maternal licking and grooming. While these disparate observations don’t allow for a simple connection to be drawn between early maternal care and playfulness they do indicate that such a relationship does exist and early post-natal social experiences can have an impact on later playfulness as a juvenile.

In order to start looking at some of these discrepancies, a crowd-sourcing approach to data collection was used in which approximately 50 students from 2 classes at Gettysburg College accessed network cameras on a regular basis to observe maternal behavior in 2 Sprague-Dawley litters that experienced a daily 15-minute period of maternal separation and 2 litters that were left undisturbed. Students in one of these classes also used the same network cameras to subsequently observe pairs of male rats from each of these litters over a 2 week period after weaning. A total of 500 discrete observations of maternal behavior were obtained for each litter and it was found that rats in the "handled" litters experienced less licking and grooming (13.2 ± 0.1% of the observations) than those in the undisturbed control litters (15.6 ± 0.4% of the observations). Despite a limited sample size, this difference was found to be statistically significant, $t(2) = 6.46$, $p = .023$. When post-weaning behavior was observed during the dark phase of the light/dark cycle (i.e., when the rats were most active) it was found that rats from the handled litters engaged in more play than rats from the control litters. Handled rats were found to be playing during 11.1 ± 0.4% of observations while control rats played during 7.5 ± 0.4% of observations. This difference was also found to be statistically significant, $t(2) = 7.01$, $p = .02$. So while these data were collected by relatively inexperienced observers and will need to be replicated under better controlled observation conditions, it does seem to suggest that Sprague-Dawley rats that are handled as infants do indeed play more when observed over a period of time in the home cage, which is consistent with past work (Aguilar et al., 2009; Siviy & Harrison, 2008), but also leaves open the possibility that brief daily separation may lead to less licking and grooming of pups. Since the handled litters in this study received less licking and grooming, these data are also consistent with other work showing that rats receiving less licking and grooming as pups play more as juveniles when tested in a home-cage environment (Moore & Power, 1992; Parent & Meaney, 2008). So while much more work is needed to clarify the precise relationship between early maternal care and juvenile playfulness, it is clear that early experiences can have a non-trivial impact on play and these experiences may specifically influence motivational variables associated with play. Taken together, these data suggest that the overall

playfulness of a rat results from a complex interplay between genetic background and early postnatal social experiences.

How do we go about mapping relevant neural circuits of play?

When attempting to map out neural circuits responsible for modulating play behavior in the rat, several strategies can be used to approach the problem. For example, one can ask what play looks like in the absence of a particular neural system or brain area. This is an approach that has been the mainstay of research in behavioral neuroscience for many decades. A particular system can be either down-regulated or up-regulated, targeting either neuroanatomical or neurochemical substrates. Brain areas can be irreversibly lesioned or temporarily inactivated by infusing compounds that will inhibit activity in that region for a limited period of time. Another approach is to ask what the brain looks like when a rat is playing. In other words, is play associated with a distinct activation of specific neural circuits? Using metabolic markers such as c-fos (Dragunow & Faull, 1989) in conjunction with well-defined and discrete behaviors can provide a remarkably detailed metabolic map of the brain as it was engaging in that behavior. Ideally, all of these approaches should result in data that converge on the same conclusions regarding the role of the targeted area or system.

Cortical modulation of play

When searching for neural mechanisms thought to be responsible for modulating play in the rat, there has been a historical and conceptual predilection to at least initially parse neural involvement into cortical and sub-cortical processes. This has been largely influenced by Paul MacLean's conceptualization of the triune brain (MacLean, 1985, 1990), with an emphasis on subcortical limbic structures as being critical for many shared mammalian-specific behaviors. If play appeared as an adaptive behavioral strategy fairly early in mammalian evolution then one would predict that neural substrates of play would primarily reside in fairly old subcortical circuitry shared by all mammals. Indeed, this was one of the first hypotheses to be tested when neuroscientific inquiries into playfulness began in the late 1970s and early 1980s. Initial studies looking at the effects of either total or partial decortication on subsequent play behavior when assessed as juveniles provided general support for the

idea that play circuitry resides predominantly within subcortical structures. Although reductions in pinning were observed in total decorticate rats when tested in like-lesion pairs, overall rough-and-tumble activity and play solicitation was not adversely affected (Panksepp et al., 1994; Pellis et al., 1992). When decorticate rats were paired with a non-lesioned control rat play was virtually indistinguishable from the control rats. Neonatal aspiration limited to the frontal cortex can even increase play solicitation when these rats were paired with non-lesioned partners or when lesions are made unilaterally (Panksepp et al., 2003; Panksepp et al., 1994). These findings were in general agreement with an earlier paper showing minimal effects on the play of hamsters as long as damage did not extend into subcortical areas (Murphy et al., 1981). Since neonatal aspiration of somatosensory cortex reduces pinning while increasing play solicitation (Panksepp et al., 1994) it is likely that the deficits in pinning following near-total decortication may be due to a disruption in the somatosensory processing that is necessary to maintain an active play bout (Siviy & Panksepp, 1987b).

While rats without a neocortex exhibit all of the elements of play behavior and in some cases play at levels that are indistinguishable from non-lesioned rats, subtle differences among decorticate rats suggests that some type of modulation of play occurs at the level of the cortex. These differences can become particularly salient when more detailed observations of play are made and when these observations are followed into early adulthood. For example, Pellis and his colleagues have compared the play of juveniles to that of young adults and have characterized age-related shifts in how intact rats respond to playful solicitations as they mature (Pellis & Pellis, 1990; Pellis et al., 1993). As juveniles, intact male rats are most likely to respond to playful solicitations by rotating completely onto their back (i.e., a pin) but as these rats mature, they are less likely to be pinned since they tend to only rotate partially, often with their hind paws still firmly planted on the ground. Females, on the other hand, will respond to playful solicitations using complete rotations with the same likelihood both before and after puberty. As adults, intact male rats also modulate how they respond to playful solicitations depending upon the status of the rat that they happen to be playing with. When playing with a dominant male,

subordinate males continue responding to playful contacts by allowing themselves to be pinned. But when paired with another subordinate, these rats respond mostly with partial rotations.

When assessing play before and after puberty, decorticate rats were more likely to respond to nape contacts with partial rotations at both ages suggesting that decorticate play in juveniles more closely resembled adult play in rats (Pellis et al., 1992). These rats also did not modulate their responses based on the status of the partner. Subsequent studies from Pellis' group found that different areas of the cortex appear to be modulating these different aspects of play. For example, rats with lesions to the motor cortex do not show age-related changes in play tactics in that males respond predominantly with partial rotations before and after puberty (Kamitakahara et al., 2007). On the other hand, rats with damage to the orbitofrontal cortex fail to modulate their play based on the status of the partner (Pellis et al., 2006). Rats with damage to the medial prefrontal cortex simply use less complex play tactics (e.g., they are more likely to run away) when solicited (Bell et al., 2009). Similar effects on adult play were observed when lesions were made after puberty suggesting that these effects are more than likely due to removing these cortical areas rather than having an adverse effect on neurodevelopmental processes.

While the lesion studies mentioned above were unlikely due to any changes in the trajectories of neurodevelopmental processes, there is always some degree of uncertainty with any permanent lesion that effects could be attributed to compensatory mechanisms resulting from total and irreversible removal of a brain structure. In order to circumvent some of these interpretational difficulties, more recent studies have looked at play in rats following acute pharmacological inactivation of relevant brain areas. By infusing compounds that act selectively on inhibitory GABA receptors it is possible to temporarily shut down functioning in a discrete brain area. For example, comparable reductions in both pouncing and pinning were found after temporary inactivation of prelimbic cortex, infralimbic cortex, or medial/ventral orbitofrontal cortex while having no effect on general activity and having either no effect on, or slightly increasing social investigation (van Kerkhof et al., 2013b). These data reinforce previous findings suggesting that prefrontal areas have a behaviorally specific positive modulatory influence on playfulness in juvenile rats.

The prefrontal cortex has received considerable attention as a putative primary site of action for the pharmacological treatment of Attention Deficit/Hyperactivity Disorder (ADHD) (Arnsten et al., 1996; Robbins & Arnsten, 2009). Since psychomotor stimulants such as amphetamine and methylphenidate are quite potent at reducing playfulness (Beatty et al., 1982, 1984; Field & Pellis, 1994) and are also beneficial in the treatment of ADHD, play could be a useful model for studying the neurobiological substrates of this disorder. As is the case with cognitive improvements following treatment with psychomotor stimulants (Arnsten et al., 1996, 1998) the effects of these compounds on play are primarily due to increased synaptic levels of norepinephrine (NE) as these reductions in play can be blocked by selective antagonists at alpha-2 receptors and mimicked by selective noradrenergic reuptake inhibitors (Achterberg et al., 2014; Vanderschuren et al., 2008). Both methylphenidate and the selective NE reuptake inhibitor atomoxetine also reduce play when infused directly into either the anterior cingulate cortex or infralimbic cortex (Achterberg, et al., 2015). This suggests that NE acting at alpha-2 receptors in these sub-regions of the prefrontal cortex inhibits playfulness. While this also suggests that prefrontal areas are the primary site of action of psychomotor stimulants in reducing playfulness, these authors also report similar reductions with methylphenidate when infused into either the basolateral amygdala or habenula. Furthermore, reductions were not observed after infusions into either the prelimbic cortex or orbitofrontal cortex, adding emphasis to the heterogeneity associated with the cortical modulation of play.

Lesion and temporary inactivation studies, along with pharmacological approaches, provide rich pictures of the relevant brain mechanisms of playfulness, yet these are still static insights into what must be a very dynamic process of interacting neural systems. This is where the use of metabolic markers, such as c-fos, have proven to be particularly useful for obtaining a more dynamic picture of these systems. While an earlier study reported no play-associated increases in c-fos expression in prefrontal areas (Gordon et al., 2002) a more recent study found enhanced c-fos expression during play in anterior cingulate cortex, prelimbic cortex, medial orbitofrontal cortex, and ventrolateral orbitofrontal cortex. Other prefrontal areas showed no increase in c-fos expression or, in the case of the dorsolateral orbitofrontal cortex, exhibited a play-associated decrease in c-fos expression (van Kerkhof et al., 2014).

Taken together, these data suggest an important modulatory role for select regions of the prefrontal cortex in play behavior.

Subcortical involvement

The studies described above indicate that play is modulated in fairly subtle ways by cortical processes and that there is considerable anatomical heterogeneity in this modulation; yet it is still likely that subcortical systems are the targets of such modulation and there are several subcortical areas that stand out as potentially important hubs in any putative play circuitry. Given the importance of somatosensory processing during rough-and-tumble play (Siviy & Panksepp, 1987b) it was perhaps not surprising to find that discrete damage limited to subcortical areas known to process somatosensory input, such as the parafascicular area of the thalamus (PFA), results in a robust, long-lasting, and selective impairment of play (Siviy & Panksepp, 1985, 1987a), without compromising complex sensory-motor processes such as foraging for food. The PFA, perhaps along with other components of the intralaminar thalamic nuclei, may then receive direct somatosensory input from the spinal cord and send excitatory projections to areas such as the frontal cortex and striatum (Cesaro et al., 1985; Nakamura et al., 2006; Voorn et al., 2004). These areas may help transduce playful somatosensory input into the fluid motor sequences seen during play. Recent evidence suggesting that PFA input to the dorsal striatum facilitates behavioral flexibility (Brown et al., 2010) may be particularly salient in this regard. Although play-induced increases in c-fos expression have been noted in midline thalamic structures (Gordon et al., 2002; van Kerkhof et al., 2014) these increases do not appear to be selective for the PFA, suggesting that input through these midline structures may be somewhat diffuse.

Given the behavioral richness and sensorimotor complexity associated with rough-and-tumble play, it would not be unexpected to find a prominent role for the striatum in the modulation of playful behaviors as well. Indeed, the relative size of the striatum within a subset of non-human primates has been reported to predict the amount of social play (Graham, 2011), suggesting that higher amounts of play in a particular species may be associated with a larger striatum. Play-induced increases in c-fos expression have been noted in both dorsal and ventral striatum (Gordon et al., 2002; van Kerkhof et al., 2014),

suggesting that both regions of the striatum are active during play behavior. Within the dorsal striatum, activity is most pronounced in the dorsolateral portion of the dorsal striatum (van Kerkhof et al., 2014) and since the dorsolateral striatum is primarily associated with somatosensory input (Voorn et al., 2004) these authors suggest that dorsal striatum may be particularly important for the spatial and temporal organization of rough-and-tumble play. While this would imply that increased activity within the dorsal striatum increases playfulness, it is interesting that blockade of excitatory glutamate receptors in the dorsomedial striatum, a manipulation that would tend to decrease excitatory input into this region, increases play (van Kerkhof et al., 2013b). So while increased activity in the dorsolateral striatum may be associated with enhanced playfulness, increased activity in the dorsomedial striatum may tend to inhibit playfulness.

Because of serious motoric deficits that can arise from lesioning the striatum, it has not been practical to examine play when the striatum has been significantly compromised. Nevertheless, neonatal 6-OHDA lesions of the striatum, which compromised dopaminergic functioning within the dorsal and ventral striatum, disrupts the patterning of play (Pellis et al., 1993). This suggests that dopamine (DA) acting within the striatum is involved in the sensorimotor organization of play. DA acting on neurons within the nucleus accumbens may also be particularly important for some of the affective qualities associated with play as well given the known involvement of the nucleus accumbens in reward-related processes (Berridge, 2007; Cardinal et al., 2002; Humphries & Prescott, 2010; Ikemoto & Panksepp, 1999; Young et al., 2011). As mentioned earlier, rats will emit high frequency 50 kHz USVs when playing (Burgdorf et al., 2008) and when anticipating play (Siviy & Panksepp, 2011). Playback of 50 kHz vocalizations also leads to increased release of DA in the nucleus accumbens as assessed by fast scan cyclic voltammetry (Willuhn et al., 2014). Similarly, social interactions can increase DA release in the nucleus accumbens of both adolescent and adult rats (Robinson et al., 2011). Yet while habituation was observed upon a second opportunity for social interaction among adults the magnitude of release was not diminished among adolescent rats. Finally, rats of the F344 strain are less playful than other strains and also release less DA in dorsal and ventral striatal slices and exhibit less DA-dependent plasticity when

assessed in striatal slices (Siviy et al., 2011). Taken together, these data strongly implicate DA acting within the dorsal and ventral striatum as being important for various aspects of play.

Opioids are also known to be involved in play behavior (Panksepp , 1985; Trezza & Vanderschuren, 2008; Vanderschuren et al., 1995a, 1995b, 1995c, 1996) and this is likely due to effects being exerted within the ventral striatum. Infusions of μ-opioid agonists into the either the core or shell sub-regions of the nucleus accumbens increase play whereas μ-opioid antagonists reduce play, block the play-enhancing effect of agonists, and prevent the establishment of a play-induced conditioned place preference (Trezza et al., 2011b). These data suggest that the release of endogenous opioids into the nucleus accumbens enhances play by modulating the reward value or emotional valence of playful social interactions. Although not directly tested yet, it is possible that the effects of DA and endogenous opioids may intersect and act in concert within the nucleus accumbens to modulate play and play reward.

The hypothalamus is a critical hub for many motivated behaviors and, as with the striatum, the size of the hypothalamus in non-human primates predicts the amount of play for those species included in this sample (Lewis & Barton, 2006). While lesion studies assessing the involvement of hypothalamic structures in play have been limited and not very informative (reviewed in Panksepp et al., 1984), other approaches provide evidence for some hypothalamic involvement in the modulation of play. Play-induced increases in c-fos expression have been reported in the ventromedial hypothalamus of rats (Gordon et al., 2002) as well as the ventrolateral hypothalamus of hamsters (Cheng et al., 2008). Play-associated increases and decreases in opioid receptor binding in hypothalamic structures have also been reported (Panksepp & Bishop, 1981; Vanderschuren et al., 1995c) suggesting that differential opioid release within the hypothalamus may help modulate levels of playfulness. Vasopressin originating from cells within the paraventricular nucleus may also be a relevant modulator of play as a positive correlation has been reported in rats between levels of play and mRNA levels for vasopressin (Paul et al., 2014). A role for vasopressin in play is supported by a finding that intracerebroventricular infusions of a vasopressin antagonist reduces play in male rats (Veenema et al., 2013) although this same study reported an increase in male rats and a decrease in female rats when the same antagonist was infused into the

lateral septal nucleus. More work addressing the involvement of hypothalamic hormones such as vasopressin and oxytocin is clearly warranted.

Another subcortical area that has drawn considerable attention has been the amygdala. As with the striatum and the hypothalamus, relative size of the amygdala within a subset of non-human primates can also predict amount of play (Lewis & Barton, 2006). Relatively large lesions to the amygdala reduce play (Daenen et al., 2002; Meaney et al., 1981) and while caution needs to be used in interpreting the behavioral specificity of these effects (Panksepp et al., 1984; Siviy & Panksepp, 2011) more recent studies have affirmed that this region more than likely has some role in the modulation of play behavior. Play-associated increases in c-fos expression have been observed in both the lateral amygdala and the bed nucleus of the stria terminalis, with play-associated activity in these areas correlating with activity in the striatum (van Kerkhof et al., 2014). While these authors also report no play-induced increase in c-fos expression in the basolateral, medial, or central amygdala, play-associated activity in these regions was found to be correlated with play-associated activity in the orbitofrontal cortex. These data suggest that during play there is coordination of neural activity between frontal cortex, striatum, and amygdala.

At a neurochemical level, increased release of endogenous cannabinoids in the amygdala may serve to facilitate play (Trezza et al., 2012). Play increases levels of the endocannabinoid anandamide in the amygdala and when the fatty acid amide hydrolase inhibitor URB597 is infused into the basolateral amygdala, thus preventing the metabolic breakdown of anandamide and prolonging the availability of anandamide in the synapse, play is increased. This suggests that play is associated with an increased release of anandamide in the basolateral amygdala and that this increased release leads to increased playfulness. Infusion of the selective CB_1 antagonist SR141716A into the basolateral amygdala reduces play by itself and can also block the increase induced by a systemic injection of URB597, demonstrating receptor selectivity of this effect. NE may also be acting within the amygdala to impact play, but in the opposite direction of endocannabinoids. Methylphenidate and atomoxetine increase synaptic levels of NE by blocking reuptake and both reduce play when infused into the basolateral amygdala. These effects have been suggested to be due to increased availability of NE acting at α_2 receptors (Achterberg et al.,

2015). So while endogenous cannabinoids can enhance play by acting on CB_1 receptors in the basolateral amygdala, NE inhibits play most likely by acting on α_2 receptors.

The data presented so far highlights the importance of prefrontal, striatal, and amygdala involvement in play. There is considerable evidence suggesting a significant modulatory influence for monoamines on play (Siviy & Panksepp, 2011; Trezza et al., 2010) and these systems have their primary cell body groups in the midbrain and hindbrain. One of the primary hubs connecting these midbrain/hindbrain structures with forebrain limbic structures is the habenula. Located along the midline and above the thalamus, the habenula has extensive reciprocal connections that bind together a variety of striatal, limbic, and midbrain structures (Bianco & Wilson, 2009; Lecca et al., 2014; Lecourtier & Kelly, 2007; Sutherland, 1982). When adolescent rats are isolated for 24 hours there is increased c-fos expression throughout the habenula that is much greater than what is observed in rats that have only been isolated for 3.5 hours (van Kerkhof et al., 2013a). When provided with an opportunity to play for 15 minutes, the isolation-induced enhanced expression is attenuated in the medial segment of the lateral habenula. This suggests that neuronal activity within the habenula may be particularly sensitive to the motivational state of the rat and influence activity in monoaminergic cell body regions that could, in turn, modulate forebrain limbic structures. These same investigators also report that temporarily inactivating the habenula reduces play. Pinning was found to be more sensitive to disruption than play solicitation (pouncing) and since pinning is thought to prolong a bout of play (Panksepp et al., 1984; Pellis et al., 1997; Vanderschuren & Trezza, 2014) these authors suggest that one function for the habenula in the young rat could be to prolong positive social interactions.

Although a fully mature model of a neural circuit for play is not yet in our grasp, we can glean enough information from the available data to begin cobbling together a tentative model for how the brain may modulate play. Examining correlations in c-fos expression between brain areas during a bout of play have been particularly insightful (van Kerkhof et al., 2014), especially when viewed alongside those studies looking at behavioral effects associated with loss of function. The initial motivation to play can perhaps be best understood by assessing the consequences of short-term isolation (e.g., up to 24 hours).

Since c-fos expression increases in the habenula after 24 hours of isolation (van Kerkhof et al., 2013a), it is likely that this area may be important when it comes to an animal being motivated to play. These authors suggest that the increased activity in the habenula following isolation could reflect a negative affective state associated with the lack of social contact and that this may also be reflected in a dampening of dopaminergic activity in the midbrain. We have observed an enhanced response to amphetamine in juvenile rats that have been socially isolated for 3 days, suggesting that meso-striatal dopamine terminal areas may become sensitized when social contact is prevented (Siviy & Panksepp, 2011). So after a period of social isolation, forebrain dopamine systems may be primed for the presence of socially salient stimuli, such as a putative play partner (Robinson et al., 2011).

Once play is in full form, coordinated activity between prefrontal areas, striatum, and amygdala may continue to modulate the unique aspects of play behavior. For example, play can be distinguished from many other motivated behaviors in the flexibility and inter-changeability among individual behavioral patterns. Correlated activity among prefrontal cortex, striatum, and amygdala, along with continued modulation arising from the midbrain monoaminergic neurons has been suggested to account for this level of flexibility (van Kerkhof et al., 2014; Vanderschuren & Trezza, 2014). In this regard, it is perhaps noteworthy that of all prefrontal areas examined, play did not increase c-fos expression in the infralimbic cortex, an area that seems to be particularly relevant for guiding behavior once a habit has been firmly imprinted (Smith & Graybiel, 2013).

Can the benefits of play be mapped onto these neural circuits?

As an organism navigates through life it constantly encounters novel and uncertain situations so being able to respond adaptively in these situations provides a significant survival advantage. Playful social interactions during the juvenile and adolescent period are thought to afford the opportunity to develop flexible behavioral strategies that can be beneficial when these types of situations are encountered in adulthood (Pellis & Pellis, 2009; Spinka et al., 2001; Vanderschuren & Trezza, 2014). For example, animals that have been deprived of the opportunity to play during a discrete period (e.g., limited to the 3rd and 4th weeks of life) as juveniles and then re-housed socially tend to respond inappropriately to

challenging social situations when tested as adults (Van den Berg et al., 1999) and also respond more impulsively when a cognitive task becomes more demanding (Baarendse et al., 2013) suggesting that play experiences may improve both cognitive and social functioning under challenging conditions.

Play-associated plasticity within the prefrontal cortex has been noted in studies looking at both play deprivation and those assessing the effects of differential play experiences on prefrontal neurons, suggesting that this area may have a critical role in modulating the beneficial effects of play. For example, Baarendse and colleagues (2013) report that pyramidal neurons in the medial prefrontal cortex in rats that had been play-deprived during the 3rd and 4th weeks of life are less sensitive to modulation by dopamine. We have also reported impaired dopamine-mediated plasticity in the prefrontal cortex of the relatively non-playful F344 rat (Siviy et al., 2011) further suggesting that play, or the lack thereof, may have an impact on prefrontal cortex functionality. Dendritic complexity of pyramidal cells within the medial prefrontal cortex has also been shown to be sensitive to play experience as a juvenile (Bell et al., 2010). In particular, there is a decrease in the complexity of dendritic branching in these neurons among rats that have been given ample opportunities to freely engage in play when compared to rats reared with a non-playful adult female. These authors suggest that play experiences lead to medial prefrontal neurons that are more efficient in processing information. This was supported by a subsequent study showing that neurons within the medial prefrontal cortex were more sensitive to nicotine-induced plasticity in animals that had prior play experience as juveniles (Himmler et al., 2013). Given the importance of the prefrontal cortex in behavioral flexibility (Ragozzino, 2007) play-induced plasticity in prefrontal functioning may then yield an adult that is better able to respond adaptively to changing environmental and social demands.

Conclusions

There is ample evidence that engaging in play as a juvenile leads to an adult that is better able to navigate an ever-changing social, emotional, and cognitive landscape (Vanderschuren & Trezza, 2014). While play behavior may not be an indispensable component of the mammalian behavioral repertoire in the same sense as other motivated behaviors such as feeding and drinking, engaging in this behavior prior

to puberty more than likely gives the organism an added edge over those that don't play. Since those brain mechanisms responsible for modulating and guiding play are probably also those that may benefit from this behavior, gaining more insight into the neural mechanisms of mammalian playfulness is likely to also add considerably to our understanding of the benefits associated with play experiences. From the data gathered so far we can conclude with a reasonable degree of certainty that areas within the prefrontal cortex, striatum, and amygdala act in a coordinated fashion to yield the rich behavioral repertoire seen during active bouts of rough-and-tumble play. It is these areas that may be the primary beneficiaries of playful experiences.

Acknowledgements

Supported by NIMH grant R15MH100585 to S.M. Siviy

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