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Deprivation of Social Play: Implications for the Mechanisms of Autism Spectrum Disorders

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Abstract

Social play emerges in early adolescence and is one of the primary types of social interaction seen early in life in mammals. The experience of social play is essential for the normal trajectory of social and cognitive development. Adolescent mammals deprived opportunities for social interaction at this age display neurodevelopmental abnormalities. Social deprivation alters adult behavioral patterns, neuroanatomy, and neurochemistry in ways that resemble autism spectrum disorder (ASD). These deficits include impairments in communication, social perception, and social behavior. However, the symptom most characteristic of the earliest stage of ASD is decreased interest in social objects. Understanding the role of early social experience, especially play, in the development of social, cognitive, and emotional functioning will provide insight into the development of ASD. In order to understand how social deprivation can affect behavior, researchers isolate animals during early adolescence. Most studies have looked at rodents since it appears that isolation-rearing of rodents has detrimental effects on social development, making it a valid model of ASD. This chapter will consider the potential of this model as a model of ASD, and how it can inform understanding of ASD and the neurodevelopmental mechanisms altered by reduced social interactions early in life.

Keywords: social isolation, autism spectrum disorder, play, ultrasonic vocalization

1. Introduction

Animal models provide a means to study behavioral and psychiatric disorders that can reveal underlying mechanisms that we are unable to address in studies in human subjects. The genetic, anatomical, biological, and behavioral characteristics of rodents (primarily rats and mice) closely resembles those of humans, making them a powerful tool for modeling psychiatric diseases [1], providing a means to examine underlying processes, as well as to test potential therapeutic approaches. Like many other conditions, attempts to develop animal models have been pursued for ASD, including those based on environmental factors such as maternal immune activation [2], as well as genetic variation [3]. The similarity between the behavioral sequelae of different types of deprivation of early social experience were long ago compared to ASD [4]. Deprivation of early social interactions, often including deprivation throughout many portions of early development resulted in impaired social and emotional functioning, and repetitive, stereotyped behavior. Over time, experimental studies that deprived animals of specific types of social experience

suggested that different outcomes result from deprivation of different types of experiences at different ages [5], indicating that specific neurodevelopmental programs produce different biological and behavior phenotypes through epigenetic programming [6, 7]. In terms of understanding ASD, this suggests that genetic contributions to ASD may act, at least in part, by altering how these epigenetic programs interact with their mediators, while experiential and environmental contributions to ASD may affect those mediators. In either case, a great deal of insight might be drawn from identifying those mediators.

Play is a behavior found throughout much of the animal kingdom [8], but differs in prevalence and complexity across lineages and even between more closely related species. Descriptions and conceptualizations of play have emphasized its apparent non-utilitarian nature, and it has often been difficult to define, but this description captures its essence: "Play is repeated, seemingly non-functional behavior differing from more adaptive versions structurally, contextually, or developmentally, and initiated when the animal is in a relaxed, unstimulating, or low stress setting" [9] Play has been broadly divided into three types: (1) solitary locomotor-rotational play, (2) object play, and (3) social play [10]. Perhaps not surprisingly, social play is most prominent and characteristic of mammals. Social play is often described as "rough-and-tumble" play, describing the physical nature of the interactions, although there are certainly other aspects of social play that are not fully captured by this description. A key point regarding definitions of play regard its seemingly non-utilitarian nature. It is in fact thought to have a purpose, and many reasons for social play have been suggested [11], that include the development of faculties necessary for successful adult life, including aspects of communication, social cognition, and emotional regulation [12]. It even has been suggested to be important in developing a moral sense of fair play [13]. This characterization is based largely upon analysis of rough-and-tumble play, one of the main types of social play in young mammals, although other types of play might certainly contribute to the development of these faculties. It is impossible to miss the correspondence between these faculties (communication, social cognition and emotional regulation) and the sorts of deficits that are observed in individuals with ASD. On this basis alone it might be thought that play (or lack of play) might have a fundamental role in the development of ASD, perhaps due to basic alterations in social motivation in ASD [14]. It must be noted that ASD may be associated with alterations to both social and non-social rewards [15].

In emphasizing the non-utilitarian nature of play, a large portion of experience is ignored that is less apparent by simple direct observation, in particular emotional and cognitive experience. Social play has somewhat obvious purposes in terms of learning about social interaction and social perception, but play still has many distinctive features that are quite different from adult behavior. This would suggest that play in not simply a "rehearsal" for adult behaviors, although it may still set the stage, in many ways, for later behavior. Play is one of the earliest non-maternally focused social behaviors seen in animals, occurring at a time when the brain is more plastic than it subsequently is in adults. Consequently, in a broader sense, one of the main functions of play may be to guide and regulate neural and behavioral development. Play is most prevalent during the adolescent phase [8, 16], although it does continue in some manner or form into adulthood. Nonetheless, adolescent play would appear to have distinct purposes in adolescence, making the peak period of play in adolescence the ideal window to study the behavior. This review will focus on play during this period, but it should be noted that once sexual maturity is reached, play behavior may be used for different purposes and is not completely absent [8, 17]. Play of other types and at other points of the lifespan are certainly important to study as well. Several reasons have been stated for the focus in this

chapter on adolescent play, but others include the consequences of deprivation of play during this time-period, which are discussed in a subsequent section.

There are various methods used to study play in rodents, but most are based on observational measures of interactions between dyads of animals. A number of factors influence play behavior, both quantitatively and qualitatively, including age, sex, novelty of the actors, novelty of the environment, etc. Moreover, one of the factors that is used to increase the motivation to engage in social play, as well as the quantity of social play, is the immediate social circumstance of the animals [18]. Social isolation for 24 hours increases the intensity of social play, and the motivation to engage in social play, and for this purpose is commonly used to study social play. However, it may also change qualitative aspects of play as well. This will be discussed in a bit more detail in a subsequent section but suffice it to say that the extent of play that is observed in any particular encounter depends on a variety of factors that would subsequently affect the response to the play experience. There are also differences in play behavior between mice and rats. This chapter will focus on social play behavior in mice and the implications of play experiences for developmental disorders, specifically when mice are deprived of social interaction.

In considering the potential implications of adolescent play for the development of social, emotional and communicative faculties, it must be stated that most play studies have focused upon physical interactions between dyads. Other behaviors that may have great importance in the development of social communication have not been as extensively explored, including vocal communications. Mice communicate with one another through the use of ultrasonic vocalization (USV). The use of USV can vary depending on the behavioral context the animal is experiencing: mother-pup retrieval [19], juvenile interaction [20], as well as opposite sex [21] and same sex [22] social encounters, that also differ according to age and other characteristics of the actors. There has been an increase in interest in understanding role of USV because of its implication in communication in mice. In the context of the present discussion it should also be added that experience with USV as a part of social interactions early in life are likely to have important developmental consequences that affect social behavior, and social perception and communication abilities later in life. Although there have been several studies conducted on behavioral effects of social isolation, very little information is known regarding how USV is affected by social isolation. Examination of this means of communication as a part of studying adolescent social encounters can potentially allow researchers to characterize the purpose of calls, both in a proximate sense in terms of from the fact effects on ongoing behavior, as well as in an ultimate (i.e. neurodevelopmental) sense in terms of its influence on the development of social abilities and social competencies.

2. Autism overview

The perspective put forward in this chapter is that the study of play, and an understanding of the neurodevelopmental consequences of play experiences, will inform our understanding of ASD, and perhaps other neurodevelopmental disorders. The incidence of ASD has risen dramatically in the last few decades, no doubt in part due to increased diagnosis, due in part to changes in diagnostic criteria between DSM-IV-TR and DSM-5 [23], and perhaps over-diagnosis, but also truly increased incidence of the disorder based on a variety of environmental factors [24]. Among these environmental factors is *in utero* exposure to selective serotonin reuptake inhibitors (SSRIs) [25], which is also seen in animal models of hyperserotonemia [26]. Increasing concern about ASD comes it is one of the fastest growing developmental disorders affecting the ability to socialize and communicate, with

devastating effects that make many individuals unable to function in society on their own. According to the Center for Disease Control and Prevention (CDC), the disorder affects 1 in 59 children, with boys being four times more likely to develop ASD compared to girls [27]. Life-long disabilities in these patients significantly impacts several areas of functioning including communication, social interaction, social perception, emotional regulation and sensory processing, and individuals with ASD often exhibit repetitive, and sometimes self-injurious, behaviors [28]. Currently there is no pharmacological treatment that specifically targets the main symptoms of ASD, although individuals with ASD are often on a variety of medications to treat various secondary symptoms [29]. Primary treatment modalities include different types of psychological and occupational therapies [30]. These therapies could certainly be complemented by effective pharmacotherapies, but they are not yet available, and even approaches to identify potential treatments are in need of development. Part of the difficulty here is the complex causal web that underlies ASD, as well as the broad spectrum of symptom occurrence and severity that occur in individual patients. The factors contributing to ASD include environmental, genetic and gene-environment interactions [31], but each particular contribution appears to be small, particularly for genetic factors. Considerable efforts are now focused on understanding the genetic causes of autism and using the genetic findings to select rational targets for effective treatments [32–34]. As with other psychiatric disorders with a complex causality, there is some thought that understanding the genetic and environmental factors contributing to the development of ASD will lead to the identification of convergence of these factors on common systems. An alternative approach is to identify the systems underlying the neurodevelopmental processes upon which genetic and environmental variation may act. Therefore, there is a need to study the neurodevelopment of social behavior to better understand the shift in brain development in patients with ASD.

One of the longest-standing debates in the field of autism treatment involves when the disorder can be initially diagnosed. Although the age at diagnosis is generally between 3 and 6 years of age, diagnoses can be made under the age of 3 [35]. Autism diagnoses and symptoms are also highly stable over time, even from an early age [36]. Given the age of developmental emergence of the behavioral functions altered in ASD, it should be expected that certain symptoms might be observable very early in life. Infants show socialization skills, and more importantly social motivation, by gazing at faces, turning towards voices, and smiling within the first couple of months of age. Children with ASD have difficulty engaging in everyday social interactions even from this early age, and show deficits in the earliest aspects of social interaction such as eye gaze [37]. Lack of response to names, reduced interest in people, and delayed speech are some early symptoms of the disorder that are observable by as early as 8 to 10 months of age. People with ASD have difficulty interpreting what others are thinking and often miss social cues such as a smile, frown, or extending an arm for a hug. Social anxiety is also common, but to a certain extent social signals are just not understood by individuals with ASD. Being unable to interpret gestures and facial expressions makes it difficult for people with ASD to see things from another person's perspective, which in turn can interfere with the ability to predict or understand a person's actions. This difficulty with emotions is also characteristic of their own emotions. It is common for those with autism to have difficulty regulating their emotions, leading to disruptive and sometimes physically aggressive behavior. Speech and gestural communication are usually delayed in children with ASD and some have difficulty forming meaningful sentences and may speak only single words or repeat the same phrases. Slight changes can be stressful on the individual which may lead to outbursts. Evaluating these symptoms early on can help alleviate the challenges the individual faces

throughout their life. The symptoms described here represent a range of psychological and behavioral functions that emerge over the first few years of life, that build upon each other to form the basis of social interactions, the core of which involve the ability to understand and communicate with others. Part of the emphasis upon early diagnosis and treatment presumes that early intervention will produce better outcomes. One reason for this assumption is that interventions alter early life social experiences which shape later behavior, and behavioral capacity.

One of the fundamental reasons for the description of autism as a spectrum is that symptoms vary substantially between individuals in terms severity, but also qualitatively, as well as having highly variable co-morbidities. Given this variability in presentation it is not surprising that ASD cannot be explained by a single underlying cause, but rather has a highly multifactorial etiology [32, 38, 39]. Although the causes of autism are not completely clear, there is evidence that environmental factors before and after birth contribute to the risk of developing ASD, including advanced paternal and maternal age, maternal illness during pregnancy, extreme prematurity, low birth weight, and exposure to high levels of pesticide and air pollution during pregnancy [40]. However, these factors by themselves do not cause autism, but rather act in combination with genetic risks [41, 42], resulting in common underlying transcriptomic changes associated with alterations in neural function and connectivity [43]. Both the genetic and the environmental (epigenetic) contributions to ASD are numerous, but as an example childhood abuse alters methylation patterns in the brain associated with epigenetic changes regulating gene expression [44, 45]. Although much of the research on early childhood trauma focusses upon stress as a causal outcome of early trauma, there is much more evidence that adverse early environments alter neurodevelopmental mechanisms associated with social experiences [7]. Of most relevance to the current discussion, evidence from the study of social isolation in experimental animals suggests that there are two developmental trajectories, one associated with social experience and one associated with its absence. Indeed, the epigenetic changes that are involved in neurodevelopmental disorders primarily affect cell differentiation, tissue specification, and cell maintenance [46]. Although much research has focused on the genetic and environmental/epigenetic factors that may contribute to ASD, much less focus has concentrated on the potential of these factors to act through underlying neurodevelopmental programs, programs that are regulated by early social experience, in particular social play.

3. Social play behavior

Long-standing interests in social play have focused on the pathological consequences of its absence. Experimental approaches to the study of social play behavior have focused on rodent models. Early studies of adolescent social deprivation demonstrated that it was specifically deprivation of play experiences that primarily drove the consequent long-term outcomes ([47], for review see [5]). Moreover, it was clear that even a short period of social deprivation increased motivation to engage in social play [48]. Indeed, this effect of isolation became a fundamental part of approaches to study adolescent social interactions. Social play, also called "rough-and-tumble play" or "play-fighting," is one of the earliest forms of non-mother-directed social behavior observed in mammals. In rodents, this occurs when one adolescent rodent grabs, holds, bites, or otherwise contacts another adolescent rodent. Unlike serious fighting, the behavior occurs in the absence of functional consequences such as resource acquisition or protection and when the needs of the animal are fully met. Although the range of play behavior is extensive, researchers generally define it as an activity that is voluntary and highly reinforcing. The suggestion that males engage in
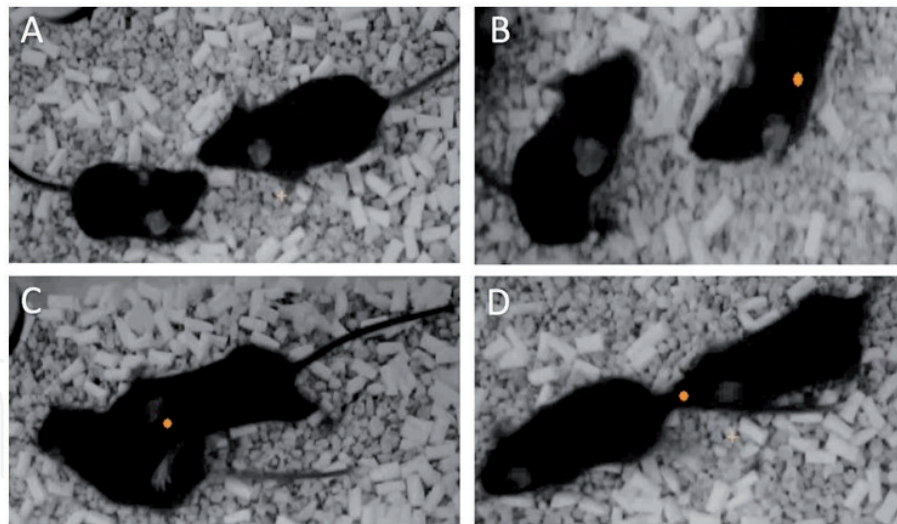
Figure 1.
Social and non-social behavior in C57BL/6 mice between 28 and 35 PND. (A) Nose to nose sniffing.
(B) Rearing (mouse on left). (C) Dorsal contact (mouse on right directed towards mouse on left).
(D) Anogenital sniffing.

social play more than females [49] results in part on an emphasis upon play-fighting, which males exhibit more frequently and for a longer duration than females. Social play is suggested to be an affiliative form of behavior functioning to facilitate social development, and the neural changes that underlie that development. Outside of its primary context, play appears to have an obvious benefit that serves to develop physical, cognitive and social capacities for adulthood [50]. The multifunctional role of play may also facilitate the development of other non-social cognitive and emotional abilities. During rough-and-tumble play, rodents engage in a series of behaviors that include dorsal pinning, nose to nose sniffing, anogenital sniffing, and following (See **Figure 1** for some examples). Some of these behaviors may produce specific types of unique sensory experiences—for example, anogenital sniffing may expose juvenile animals to pheromones. Much focus has been on other types of behavior that may be related to dominance—and consequently more male oriented, such as dorsal pins. This might help to create the impression that males engage in more play, but in fact this may come from the definition of "play". In any case, with regard to dorsal pins and similar types of juvenile "wrestling"-like behavior, there will be circumstances where the winners and losers become more consistent until they assume distinct phenotypes that researchers can categorize as "dominant" or "subordinate" [7]. There is some doubt as to whether this necessarily translates into adult forms of dominance behavior. Nonetheless, there has been substantial emphasis on this type of behavior in play research. Graham and Burghardt [8] categorized play behavior based on five criteria:

1. It is incompletely functional in the context in which it appears.

2. It is spontaneous, pleasurable, rewarding, or voluntary.

3. It differs from other more serious behaviors in form (e.g., exaggerated) or timing (e.g., occurring early in life before the more serious version is needed).

4. It is repeated, but not in an abnormal or unvarying stereotypic form (e.g., rocking or pacing).

5. It is initiated in the absence of stress.

Again, it is important to note that play behavior is sexually dimorphic in rodents (and other mammals), with males exhibiting more "rough-and tumble" behavior compared to females. This does not mean that females do not play, but rather that they play differently. Females significantly spend more time exploring than males and are more likely to engage in nonsocial behaviors [51]. In addition, it is also suggested that females investigate and approach their partners more than males. Males, on the other hand, spent the majority of their time in close physical contact with play partners. Although sex differences have been noted the majority of animal research studies investigating play have focused on males. Single-sex studies in males have outnumbered single-sex studies in females by a ratio of 5.5:1 [52]. Since play behavior is different in males and females, it is quite likely that it helps shape the brain and behavior in different ways, so that the consequences of the loss of social play are different.

Depriving young animals of maternal and peer social contact has long-lasting effects that persist into adulthood [53–56]. Indeed, it is perhaps of fundamental importance to determine whether the consequences of different types of early life experience are permanent. If such effects are deleterious, either in the sense of creating abnormal behavior, or in terms of preventing the development of certain types of behavior, it might be essential to intervene early in life. This description certainly fits ASD and other developmental disorders of childhood, where it has long been recognized that earlier interventions produce better outcomes. This is certainly true for language function, which has absolute critical periods. There is an intense period of social play in adolescence that is often depicted by an inverted U-shaped curve. Social play is most prominent in adolescent rodents and the behavior declines as they approach sexual maturity (**Figure 2**). Other behaviors certainly emerge at this time but have been less well-studied compared to either early adolescence or adulthood. Based on the viewpoint that play and other social behaviors early in life constitute a "dress rehearsal", so to speak, for adult behaviors, much research has focused on how adolescent play experiences might enhance or diminish social competency and performance later in life [57], in particular mating and dominance behaviors.

Social approach is at the core of virtually all social interactions, including those between animals of the same sex and different sexes, and between familiar and unfamiliar animals. Indeed, social approach can provide important information about the relationship of animals involved in a social interaction. For example, in some situations the social approach is associated with a functional outcome (e.g. mating), while others the motivation to approach a conspecific is independent of a specific benefit [58]. There are two primary hypotheses as to why animals play: (1) it occurs because it is intrinsically rewarding in and of itself (and only occurs if the animals are happy and stress free) and (2) it occurs because it offers some type of beneficial outcome such as refinement of motor skills. However, the two hypotheses are not mutually exclusive [59], and the
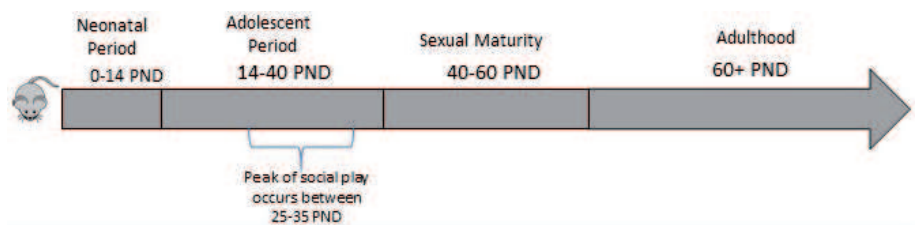
Figure 2.
Developmental periods in mice noting the peak period of social play during adolescence, occurring between 25 and 35 days of age.

benefits of play may depend on developmental stage (e.g. pre-weaning, post-weaning, juvenile, early-adolescent, late-adolescent) and might consequently result in different outcomes.

4. Social deprivation/isolation

The opportunity to engage in social play is essential for social and cognitive development in animals and those deprived of the interaction display neurodevelopmental consequences [60]. Social deprivation produces alterations in adult behavioral patterns, neuroanatomy, and neurochemistry [61]. The most common approach used to study the consequences of deprivation of early social isolation involves permanent isolation from weaning, or isolation rearing. Before 21 days of age, rodent behavior is largely dependent on the mother; however, after weaning it becomes primarily focused on same-sex conspecifics. Researchers often perform experiments after weaning and during the juvenile period, between 21 and 40 days of age. Some effects of isolation rearing include weight gain and enhanced aggression [7], which might be thought to be characteristic of dominant animals, as if this is a type of default developmental trajectory. However, many other behavioral outcomes are observed, that include locomotor hyperactivity [62], increased exploratory tendencies (or decreased habituation) [63], and impairment of pre-pulse inhibition [64]. These behavioral changes are thought to be indicative of enhanced dopamine function [7]. Other changes include increased anxiety [65] that may be indicative of reduced serotonin function. Many other changes have been observed in isolation reared rodents (for review see [5, 7]). Many of these changes have been shown to be permanent, persisting in adulthood, and developmentally specific, only occurring after social isolation in adolescence. Although some data indicates that male mice are more susceptible than females to neurodevelopmental interference induced by early social deprivation [66, 67], this may be in part because of a focus on behavioral outcomes that are more relevant to males (as well as a simple failure to study females, and especially to compare the effects of adolescent social deprivation in males and females in the same study under the same experimental conditions).

5. Role of serotonin in social isolation and social play

Serotonin neurons are well known to be critical regulators of mood and many other functions that are disrupted after isolation rearing. Consequently, it is not surprising that chronic social isolation from weaning [68] (for review see [69]), as well as deprivation solely during adolescence followed by subsequent social housing [70], disrupts serotonin function in a variety of ways. Broadly speaking, this can be characterized as reduced serotonin function as determined by tissue serotonin levels or the ratio of tissue serotonin to 5-hydroxytryptamine levels [71, 72], but also as reduced serotonin release as determined by *in vivo* microdialysis [68, 73, 74]. Generally speaking this might result from adaptations resulting from elevated serotonin function early in life. Consequently, it has been suggested that dorsal raphe nucleus, a major site for the origin of forebrain serotonin projections, is less responsive after adolescent social deprivation [75]. The adaptations are quite diverse, and include reductions in the expression of numerous 5-HT system-related genes in the prefrontal cortex of isolation-reared mice (including 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2C, 5-HT3A, 5-HT6, and 5-HT7 receptor genes) [76]. These effects were region-dependent, the hypothalamus and midbrain having more restricted reductions, while 5-HT6 gene

expression was up-regulated in the hippocampus. The disruption of serotonin in different regions of the brain suggests that serotonin functions are not altogether reduced, but rather there are shifts the responsiveness of different components of the serotonin system. The origins of these disruptions in serotonin systems are unknown, and there is quite likely to be a complex interplay with changes in other systems that are also affected by adolescent social isolation, in particularly dopaminergic changes [77]. However, serotonin alterations are likely to play some type of primary role in the effects of adolescent social isolation since it has a role in adolescent play behavior.

Serotonin has also been shown to be fundamentally involved in play behavior [78, 79], but these effects have been suggested to be part of a broad modulation of social behavior [80]. Nonetheless, genetic or pharmacological treatments that elevate serotonin levels reduce social play in adolescent rats [81]. Similar effects were seen to occur after prenatal fluoxetine exposure [82]. Given the data on social isolation that was previously discussed, this raises questions about whether such effects entirely result from elevations in serotonin function, or perhaps result from adaptations to elevated serotonin function. It has been suggested, however, that the consequences of elevated serotonin may be bidirectional [83]. This observation might reflect the degree of perturbations in serotonin function, in a manner similar to serotonin depletions with 5,7-dihydroxytryptamine [84]. Indeed, moderate depletions produced elevations in basal extracellular serotonin levels and increased anxiety, while more severe depletions reduced extracellular serotonin levels and reduced anxiety.

Loss of the opportunity to play is thought to be central to the effects of adolescent social deprivation [47], and results in an increased motivation to engage in play in adolescence [48, 85]. However, as adults, isolation-reared rodents demonstrate a variety of deficiencies in social behavior that include impaired social recognition [86]. Indeed, the broad social incompetence of isolation-reared rodents may lead to aggression being directed towards them under ethologically relevant conditions because they do not respond appropriately during social encounters [87]. The potential comparison to ASD should be obvious, and, moreover suggest that many of the social impairments in ASD result in a similar manner, from the lack of social experience early in life, which drives the development of underlying biological mechanisms that support appropriate social behaviors. Another similarity is also found between the broader behavioral impacts of isolation-rearing upon anxiety [88] and stereotyped behavior [89], both of which are characteristic of ASD.

Perturbations in serotonin function have also been described in ASD. It has been repeatedly found that hyperserotonemia, an increased level of serotonin in the blood, is reported in 30% of autistic patients [90, 91]. The causes of hyperserotonemia, and whether this reflects similar changes in central serotonin function in this wider population of ASD patients are not known, but it is known that serotonin (5-HT) transporter gene (SLC6A4; SERT) variants modulate SERT reuptake function, thereby influencing the occurrence of hyperserotonemia in some autistic patients [92]. Research examining hyperserotonemia in ASD patients highlights the importance of classifying study groups for cognitive impairment, age, and pubertal status. For example, it was found that autistic children during pre-puberty showed a significant elevation in plasma 5-HT compared to healthy controls, whereas plasma 5-HT was not significantly elevated in a post-pubertal autistic group compared to control subjects [93]. Although peripheral 5-HT dysfunctions are consistently found in ASD patients, the biological traits that underlie these changes and how these changes may be connected to brain development and brain function have yet to be determined. Different underlying causes, both genetic and environmental, might ultimately contribute to this outcome. However, it has already been noted that genetic impairments in SERT function are observed in at least some patients

with ASD [92]. Additionally, decreased binding affinity for SERT has been observed in the brains of adults with ASD [94]. In confirmation of this relationship, ASD-related social deficits are observed in SERT heterozygous (SERT +/−) and homozygous (SERT −/−) knockout mice [26]. Deficits were observed in a standard social interaction task, as well as the social preference task. These deficits were more severe in SERT −/− mice than in SERT +/− mice, but social impairments were observed in both groups compared to littermate controls (SERT +/+) mice. Furthermore, it is interesting to note that although both SERT +/− and SERT −/− mice both had deficits in social behavior, only SERT −/− mice had increased anxiety behavior in standard tests. This would seem to suggest that these mice, in some respects, may reflect the severity of ASD, with a spectrum of effects that involve only social behavior in less severe cases, but includes more and more psychiatric co-morbidities and cognitive impairment in more severe cases. If these changes in serotonin function are critical mediators of social impairments, modulating serotonin function should improve ASD symptoms. This might be accomplished either by reducing serotonin release or by inhibiting serotonin receptors. As proof of this principle, social deficits were reversed in SERT +/− and SERT −/− mice by reducing intake of dietary tryptophan, the essential amino acid precursor of serotonin. Extracellular 5-HT levels in the brain were also decreased.

6. Communication

Given the importance of communication deficits in ASD, it would be important to explore such deficits in animal models that might reflect aspects of ASD, such as SERT +/− or SERT −/− mice, or animals that have been socially isolated. Animals use species-specific vocalization to communicate information to one another regarding identity, (individual or group), group status or mood (dominance, submissive, fear, or aggression), their next likely behavior (approach, flee, play, groom, etc.), environmental conditions (presence of predators or location of food), and in mother-offspring interactions that are likely to be involved in many roles in development, as well as facilitating maternal care. Different types of information can be conveyed through vocalization and inform us of the animal's physical, environmental, or social condition. The acoustic signal can range from simple to complex tonal signals depending on the animal's situation. Information appears to be conveyed both by the frequency of sounds, as well as the tonal pattern. Each species emits sounds in a different frequency range that is attuned with their hearing abilities. Ultrasonic vocalization is a fundamental social behavior in rodents, and understanding its evolution, and genetic and neural mechanisms will provide researchers more insight about its role in animal behavior.

Based on the use of USV to communicate, in particular during the neonatal and adolescent periods of life, mice could be a potential model for the genetic basis of human communication disorders such as autism, but to increase the utility of such studies, the role of specific aspects of USV in communication and behavior must be determined. Social deprivation produces changes in USV that seem to be related to alterations in social behavior [95–98]. Rodents are known to emit a diverse range of USV depending on the social context. When isolated from the nest, pups emit a USV that stimulates the mother to retrieve the pup [99]; this suggests that this communication signal serves a specific purpose [100]. These USVs are typically characterized by a frequency of 30–50 kHz and a duration of 10–200 ms [98], and are often termed "distress" calls, which may underestimate their purpose and meaning. As the study of USVs is still an emerging field, approaches to categorizing vocalization patterns are still being developed, but most approaches are based

upon the shape of USV calls. Each call has a name based on its shape: ascending, descending, inverted u-shape, flat, modulated, complex, and frequency "jumps". A common approach is to group the calls based on sequence (bouts), shape, frequency (50 vs. 70 kHz), duration: 5-10 ms (short), or 20–50 ms (long), or by behavioral contexts: vocalization during mating, isolation, restraint, and so forth [101]. Given the number of categorizations involved in USV, there needs to be a standard way of classifying the measurements since it appears that various articles categorize the calls differently. This consensus has yet to completely emerge. Examples of USVs from dyadic encounters between pairs of wildtype C57BL/6 J mice and dopamine transporter heterozygous knockout mice are shown in **Figure 3**. Differences based on sex and genotype are clear, but most USV types are clearly evident.

There is evidence that mouse vocalizations are associated with specific affective states such as distress or pleasure, and that these features can be sensitive to differences in specific genetic variants or the broader genetic background. Mice vocalize in the range of frequencies that extend from human-audible range (squeaks that can be heard when handling mice) and higher into the ultrasound range, above the limit of human hearing (20 kHz and higher). While rats clearly emit ultrasonic vocalizations in response to aversive and rewarding stimuli, USV in mice are not correlated with aversive or positive state, but rather may be more likely to facilitate or inhibit social interactions [102].

Adult male mice use USV for courtship behavior in order to attract or maintain close contact with a female to facilitate mating. Both 70- and 40-kHz USVs have been found to be associated with courtship, but 40-kHz calls were also observed during mounting behavior [103], as were 50 kHz calls [95]. During male-male interactions USV is most closely related to social investigation prior to any closer agonistic or aggressive encounters [104]. More details on the information content and roles of USV in social encounters are certain to emerge. For instance, it has been suggested that USVs in adult male mice are organized as a sequence of multisyllabic call elements (or syllables) similar to songbirds [105]. The multisyllabic repertoire might allow the emitter to combine and organize syllables in different ways to increase the potential information carried [21, 106], giving mice the ability to change the relative composition of syllable types during quickly changing social encounters [103, 107, 108].
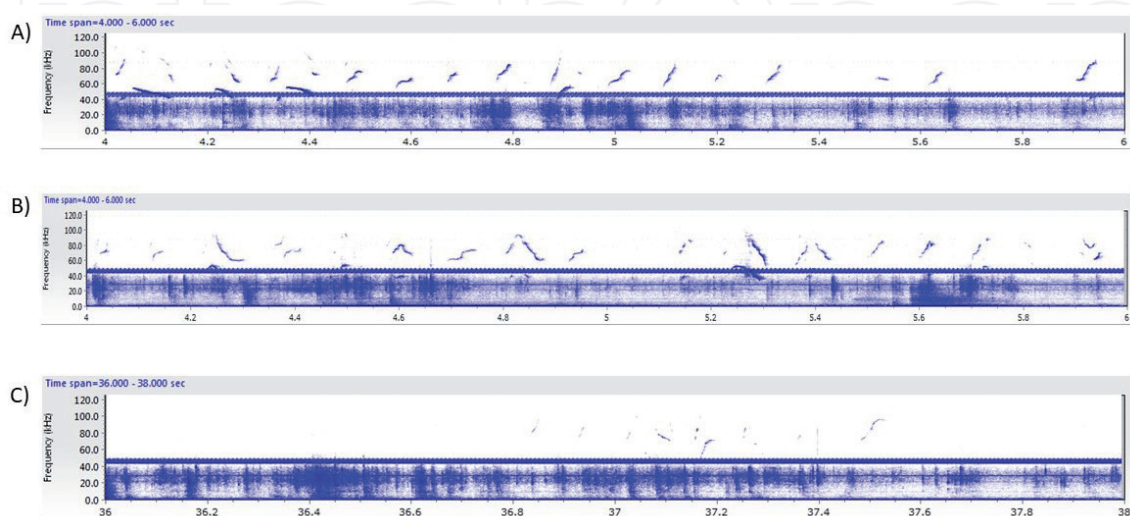
Figure 3.
Ultrasonic vocalizations emitted during social interactions between mouse dyads: an isolate mouse (deprived of social interaction for 24 hours) and a social mouse. (A) Male dopamine transporter +/− pairing. (B) Female wildtype pairing. (C) Female dopamine transporter +/− pairing. Difference are seen resulting from sex and genotype.

Although not studied as often as males, the USV emitted by female mice has a similar complexity to what has been observed in males, suggesting an important role in communication. It has been observed that female mice spend more time close to males that vocalize compared to devocalized mice, suggesting that the calls are a component of male courtship behavior [108], or perhaps a "pre-courtship" behavior that initiates proximity. Another study suggests that the USVs are emitted more during female-female interaction than in opposite-sex encounters, potentially contributing to the establishment of dominance hierarchies [109]. A considerable number of 70 kHz calls are found to be emitted by female-female dyads during social encounters, suggesting that it may be modulated by the motivational state of the emitter [110]. Regrettably, the function of USV in females are still poorly understood and further research is needed to improve our understanding of the roles of USV in female mice.

Perhaps of even more importance for the current discussion, there has been no investigation of vocalization in adolescent mice during the period of most relevance to ASD. Particularly since vocalizations in adults often appear to facilitate or initiate social interactions and may also reflect the motivation to engage in social interactions, this would be of especial importance. In studying adolescent mice new patterns and functions of USV are likely to emerge. Moreover, it will be particularly important to establish the relationship between USV and ongoing behavior. Although there are distinct patterns within USV emissions in many circumstances, the correlation of the calls to specific behaviors have yet to be determined with any degree of specificity. Varied USV patterns and bouts observed in USV emission have also made the interpretation of the calls difficult at times. There are few articles addressing the limitations of studies that involve USV calls when two or more animals are placed within a cage; it is impossible to determine which animal of the two is emitting the USV under social conditions. Establishing methods for doing this will be of critical importance for advancing the field.

7. Conclusion

The effects of social isolation have been studied for decades and although there is a quantitative way to analyze behavioral aspects of social play, the implications of USV during social interaction is still an emerging field with limited data. Ultrasonic vocalization quantification will provide us with insights on how communication is affected during social play, and how this is affected by social isolation and other models that may inform us about the underlying mechanisms of ASD. Current approaches to studying dyads of mice with different attributes (e.g. experiences or genetic differences) are certainly informative, but future studies will need to address the challenge of identifying individual mice emitting USV during social encounters.

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