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Research report

Mean girls: Sex differences in the effects of mild traumatic brain injury on the social dynamics of juvenile rat play behaviour

R. Mychasiuk*, H. Hehar, A. Farran, M.J. Esser

Alberta Children's Hospital Research Institute, University of Calgary, Faculty of Medicine, Heritage Medical Research Building, Room 277, 3330 Hospital Drive NW, Calgary, AB T2N 4N1, Canada

HIGHLIGHTS

- Clinical studies indicate that children with TBI are at risk for social impairment.
- Juvenile rats that experienced a mild TBI exhibit altered play behaviour.
- Sham animals are less likely to initiate play with a cage-mate that had an mTBI.
- Female rats with mTBI fare worse than mTBI males and are more often rejected.
- Juvenile play may be associated with lingering mTBI symptoms and long-term risk.

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ABSTRACT

Clinical studies indicate that children who experience a traumatic brain injury (TBI) are often the victim of peer rejection, have very few mutual friends, and are at risk for long-term behavioural and social impairments. Owing to the fact that peer play is critical for healthy development, it is possible that the long-term impairments are associated not only with the TBI, but also altered play during this critical period of brain development. This study was designed to determine if social dynamics and juvenile play are altered in rats that experience a mild TBI (mTBI) early in life. Play-fighting behaviours were recorded and analyzed for young male and female Sprague Dawley rats that were given either an mTBI or a sham injury. The study found that the presence of an mTBI altered the play fighting relationship, and the nature of the alterations were dependent upon the sex of the pairing and the injury status of their peers. Sham rats were significantly less likely to initiate play with an mTBI rat, and were more likely to respond to a play initiation from an mTBI rat with an avoidant strategy. This effect was significantly more pronounced in female rats, whereby it appeared that female rats with an mTBI were particularly rejected and most often excluded from play experiences. Male rats with an mTBI learned normal play strategies from their sham peers (when housed in mixed cages), whereas female rats with an mTBI show heightened impairment in these conditions. Play therapy may need to be incorporated into treatment strategies for children with TBI.

1. Introduction

Research has demonstrated that children who suffer from a traumatic brain injury (TBI) are at risk of experiencing long-lasting behavioural and psychosocial difficulties [1–4]. Difficulties in these domains likely arise from TBI induced disruptions to typical development of brain regions involved in social and emotional functioning [3]. Socio-emotional maturation spans the entire developmental continuum, from childhood through adult life [5–7]. The neurobiological processes that make up the 'social brain' similarly undergo protracted maturation and are comprised of a complex network of brain regions including the prefrontal cortex, temporal lobes and the limbic system [8–11]. Juvenile play has been identified as one of the key mechanisms involved in healthy development of the social brain [12,13] and many studies involving humans and rodents have illustrated the negative consequences associated with limited or abnormal play exposure in childhood [14–16].

Juvenile play is believed to be multifunctional, conferring more than one adaptive benefit to the participant, which may present immediately or in the long-term [12]. The benefits associated with childhood play include assessing and interacting with social partners, understanding social hierarchies and emotional communication, as well as increased behavioural flexibility. Although outcome measures following brain injury have generally been

* Corresponding author. Tel.: +1 403 210 8571; fax: +1 403 210 9685.
E-mail address: rmmychas@ucalgary.ca (R. Mychasiuk).

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restricted to functional assessment of cognitive or emotional impairment, recent studies with human cohorts have indicated that social interactions between children who have had a severe TBI and their peers may be disturbed [1]. Human studies indicate that despite average intelligence, children with TBI have few mutual friends, are often the victim of rejection, and display significant impairment in circumstances that require understanding of another's *theory of mind* [17]. This finding has many implications not only for healthy socio-emotional functioning, but it may also impede or alter therapeutic and rehabilitation strategies

The few human studies that do examine social interactions tend to study children with moderate to severe TBI despite the fact that the majority of childhood TBI is classified as mild (mTBI) [18]. Childhood is characterized by a particularly high risk for mTBI and concussion from falls, automobile accidents and sports related injuries. Furthermore, injuries arising from mTBI and concussions tend to preferentially involve the frontal and temporal lobes [19–21], affecting regions identified as part of the social brain, thereby potentially impacting neurodevelopment during critical periods. Therefore, the purpose of this study was to determine whether social interactions, specifically play behaviour, in juvenile rats that experienced an mTBI are altered or affected. The study sought to investigate whether an mTBI in the juvenile period (P30) alters the social interactions and play dynamics and whether this change was relative to the status of the play participants? In other words, does the induction of an mTBI negatively affect the social dynamics, whereby animals with a sham injury play differently with mTBI animals than they do with other sham-injured animals, and vice versa? As play is critical for normal developmental processes, alterations to the social dynamics during this time period could further exacerbate TBI symptomology and alter long-term outcomes.

2. Materials and methods

2.1. Subjects and mTBI procedure

All experiments were carried out in accordance with the Canadian Council of Animal Care and were approved by the University of Calgary Conjoint Faculties Research Ethics Approval Board. Forty Sprague Dawley rats were housed in groups of 4 and maintained on a 12:12-h light:dark cycle in a quiet temperature controlled (21 °C) husbandry room with access to food and water ad libitum.

When animals reached P30, half of the animals received an mTBI, similar to that described by Kane et al., [22], and the other half received a sham injury. Animals were lightly anesthetized and placed chest down upon a stage consisting of a slit piece of aluminium foil suspended 10 cm above a sponge cushion. A weight of 150 g was tethered with fishing line and dropped from 0.5 m through a PVC guide tube (20 mm diameter × 1.5 m length). The opposite end of the line was attached to the top of the tube to ensure that the weight did not travel beyond 1 cm after contacting the dorsal surface of the head and thus could not produce "re-hits" to the head or body of the rat. Upon impact from the weight, the rat fell through the slit tinfoil and underwent a complete 180° horizontal rotation before it fell freely onto a sponge cushion (see Kane et al. [22] for video representation). The rotation and free fall is associated with acceleration/deceleration forces to the head that are typically found in mTBI/concussion. Immediately after impact, topical lidocaine was applied to the head of the rat and it was placed in a clean cage on a warm heating pad to recover. Animals experiencing a sham injury were lightly anesthetized, placed chest down on the aluminium foil stage, but were removed without the impacting weight or the rotational free-fall. They also received application of topical lidocaine and were placed in clean cages on warm heating pads to recover. The time each rat took to right itself was recorded; *time-to-right*. Once the rats were behaving in a typical manner (walking, exploring, etc.) they were returned to their home cages. As previously described by Mychasiuk et al., [23], animals who experienced an mTBI display other clinically relevant symptomology of mTBI/concussion such as beam walking foot slips, see below. The time-to-right and beam-walking task were used in this study to validate that the modified weight drop technique produced an mTBI.

2.2. Behavioural/social play testing

Following injury, animals were subjected to the social play fighting paradigm (6 days post-mTBI) and a beam-walking task (7 days post-mTBI).

2.2.1. Social play fighting – paradigm 1 (mixed cage)

The play-fighting paradigm was set up in accordance with the procedures outlined by Himmler et al. [24] to maximize the play pairings that could be examined; therefore each cage of 4 animals was organized so it would contain two mTBI rats and two sham rats. This ensured that all play partnerships could be investigated in each home cage (mTBI + mTBI, mTBI + sham, and sham + sham). Juvenile play behaviour was tested on P37, one-week post-TBI. Twenty-four hours prior to testing, animals were removed from their home-cages where they were housed with 3 other juvenile rats and isolated in a standard shoe-box cage to increase the frequency of play in the testing period [25]. At this time point one of the play partners was colour marked on the tail so the two rats could be differentiated during scoring. On the testing day, two rats, from the same home-cage, were placed in the testing arena (50 cm × 50 cm × 50 cm) and their behaviour was recorded for 10 min in the dark with a night vision camera. Following the 10-min session, rats were returned to their home cage and the testing arena was cleaned with Virkon. The video recordings of the social play fighting were scored frame by frame by two independent researchers blinded to the experimental manipulations. On the rare occasion that the two researchers generated different results, the two researchers scored the video together to discuss the rationale for their observed results and the values from the mutual scoring was used. The animals were individually scored for the number of attacks they made to the other rat's nape, and their defensive response to an attack; complete rotation, partial rotation, horizontal rotation, evasion, and no-response [26]. When two rats play fight, the pair competes for access to their partner's nape (an attack or play initiation), if they reach the nape, the rat will gently nuzzle its partner. Following an attack, the responder who is trying to protect his or her nape, can respond with a variety of defensive manoeuvres which include; evasions (swerving, leaping or running away), complete rotations (rolling over into a supine position which leads to the rat being 'pinned'), partial rotations (rolling onto their side), and horizontal rotations (both rats stand on their hind legs and "fight" with their fore paws). If a rat does not want to play with the other rat, it can also just ignore the play initiation with a non-response (simply turning its head away from the partner) [12].

2.2.2. Social play fighting – paradigm 2 (heterogeneous cage)

As juvenile play is a form of social learning, we hypothesized that the injury state of the individual's cage-mates may influence their play behaviour. For example, a sham rat that lived with an mTBI rat for a week prior to testing (as occurred in paradigm 1) could possibly display different behaviours than a sham rat who had no prior experience with mTBI rats and vice versa. Therefore, for the second social play-fighting paradigm, rats where again housed in cages of 4, however, all four rats had the same injury (4 mTBI or 4 sham/cage). All of the procedures were repeated exactly as described above, except for the housing arrangements. Juvenile play fighting was