

# Studying the Neurobehavioral Mechanisms of Social Behavior in Adolescent Rats

L.J.M.J. Vanderschuren<sup>1,2</sup>, E.J.M. Achterberg<sup>1</sup>, P.J.J. Baarendse<sup>2</sup>, R. Damsteegt<sup>2</sup>, L.W.M. Van Kerkhof<sup>2</sup>,  
V. Trezza<sup>3</sup>

<sup>1</sup>*Department of Animals in Science and Society, Division of Behavioural Neuroscience, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands. l.j.m.j.vanderschuren@uu.nl*

<sup>2</sup>*Rudolf Magnus Institute of Neuroscience, Department of Neuroscience and Pharmacology, University Medical Center Utrecht, Utrecht, The Netherlands*

<sup>3</sup>*Department of Biology, University "Roma Tre", Rome, Italy.*

## Social play behavior

In between weaning and puberty, the young of all mammalian species, including humans, display a characteristic form of social interaction known as social play behaviour or rough-and-tumble play. This form of social behaviour is highly rewarding and essential for the development of social and cognitive skills [2,5,6,7,9,19]. Our research focuses on elucidating the neural and behavioral underpinnings of social play behaviour in adolescent rats, and its role in behavioural development. To that aim, we have developed methods to study the performance of social play behavior, the rewarding properties of social play behavior and the motivational properties of social play.

## Assessment of different behavioral components of social play behavior

The performance of social play behavior is studied in dyadic encounters. Rats (25-40 days old), that are matched for sex (we usually use males) and weight are used. To preclude the influence of existing dominance hierarchies on social play, cage mates are never tested against each other. To enhance the motivation for social behavior, rats are briefly socially isolated before the experiment. Isolation for up to 24 hr causes an orderly increase in social play behavior during the test [4,18,20]. To enable the ready detection of both increases and decreases in social play behavior, an isolation time is used that evokes a half-maximal increase in social play behavior, i.e. 3.5 hr [4].

In rats, a bout of social play behavior starts with one rat soliciting ('pouncing') another animal, by attempting to nose or rub the nape of its neck. The animal that is pounced upon can respond in different ways: if the animal fully rotates to its dorsal surface, 'pinning' is the result. Pinning is regarded as the most characteristic posture in social play in rats. As rats mature into adulthood, the structure of social play changes. Most prominently, the response to pouncing with full rotation to the dorsal surface (resulting in pinning) occurs less and is replaced by evasions and partial rotations [6]. The following behaviors are relevant measures of social play in rats [6,9]:

**Pouncing:** Nuzzling the nape of the conspecific's neck with the tip of the snout followed by a rubbing movement.

**Evasion:** Upon solicitation, the recipient animal avoids contact with the nape by leaping, running, or turning away from the partner.

**Partial Rotation:** Upon contact of the nape, the recipient animal begins to rotate along its longitudinal axis, but then stops and keeps one or both hind feet firmly planted on the ground.

**Pinning:** Upon contact of the nape, the recipient animal fully rotates around the longitudinal axis of its body, ending in a supine position with the other subject standing over it.

**Boxing/Wrestling:** Rearing in an upright position towards the other subject combined with rapidly pushing, pawing, and grabbing at each other, or wrapping around the other subject.

**Following/Chasing:** Moving or running forward in the direction of or pursuing the other subject, who moves away.

In addition to these measures, social exploration (sniffing, licking or grooming any part of the body of the test partner, including the anogenital area) is assessed as a measure of general social interest, which may not necessarily be associated with playful social behavior. Also, non-social exploratory behavior is measured, in order to detect any general behavioral impairments as a result of neural or pharmacological manipulations.

To understand the behavioral structure of social play in more detail, we have also established setups to study specifically the rewarding and motivational properties of social play. The rewarding properties of social play behavior are assessed using a place conditioning setup [1,15]. In place conditioning experiments, an apparatus is used that consists of two chambers that can be clearly distinguished by the animals, for example on the basis of different visual, tactile and olfactory cues. Preferably, the animals should have no innate preference for one of the two chambers before conditioning starts. During conditioning, the animals are allowed to engage in social play behavior in one chamber and are placed alone in the other chamber, in alternating sessions. In this way, the cues in one chamber gain meaning to the animal because they get paired with something behaviorally relevant, i.e. social play. During testing, the animals can freely move around the two chambers and the time they spend in either one is recorded. If they spend more time in the chamber that has been associated with social play, than this is said to evoke conditioned place preference, which is interpreted as social play having positive subjective ('pleasurable') effects. In order for social play behavior to evoke conditioned place preference, our parametric analysis has indicated that social isolation during conditioning and testing (causing the animals to be able to engage in social play only during conditioning sessions) is optimal. We have found that 8 conditioning sessions, lasting 30 min each, induces robust, social play-induced conditioned place preference [11].

At this moment, we are also developing an operant conditioning setup, in which animals have to lever press in order to gain the opportunity to engage in a short episode of social play. Social isolation during training sessions causes animals to readily acquire the task. Once trained, the animals perform in a stable fashion under both fixed ratio (where a fixed number of lever presses throughout the test session is required to receive the reward) and progressive ratio schedules of reinforcement (where an increasing number of lever presses after each reward is required).

## **The neuropharmacology of social play behavior**

Our recent work has focused on the neurobiology of social play behavior. These studies have revealed important roles for cannabinoid, opioid, dopaminergic, noradrenergic and serotonergic neurotransmission in social play [3,8,12-14,20, for reviews see 9,17]. This is in keeping with the rewarding properties of social play, as these neurotransmitter systems have been widely implicated in the positive subjective properties of food, sex and drugs. In-depth analysis of the underlying neural substrates has identified the nucleus accumbens as a site of action for the stimulating effects of mu-opioid receptor agonists on social play behavior [10]. Furthermore, analysis of immediate early gene expression patterns after social play revealed activation of limbic corticostriatal pathways during social play. Based on these data, pharmacological inactivation studies have subsequently confirmed the importance of the prelimbic cortex, medial orbitofrontal cortex and nucleus accumbens core for social play [16].

## **Conclusions**

Our studies have shown that the performance of social play, as well as its rewarding and motivational properties can be readily assessed in adolescent rats. Interacting opioid, cannabinoid and dopaminergic systems within the corticolimbic circuits underlying incentive motivation and reward modulate social play behaviour.

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