Serotonin-related rodent models of early-life exposure relevant for neurodevelopmental vulnerability to psychiatric disorders

Tamara S. Adjimann, Carla V. Argañaraz and Mariano Soiza-Reilly

Abstract
Mental disorders including depression and anxiety are continuously rising their prevalence across the globe. Early-life experience of individuals emerges as a main risk factor contributing to the developmental vulnerability to psychiatric disorders. That is, perturbing environmental conditions during neurodevelopmental stages can have detrimental effects on adult mood and emotional responses. However, the possible maladaptive neural mechanisms contributing to such psychopathological phenomenon still remain poorly understood. In this review, we explore preclinical rodent models of developmental vulnerability to psychiatric disorders, focusing on the impact of early-life environmental perturbations on behavioral aspects relevant to stress-related and psychiatric disorders. We limit our analysis to well-established models in which alterations in the serotonin (5-HT) system appear to have a crucial role in the pathophysiological mechanisms. We analyze long-term behavioral outcomes produced by early-life exposures to stress and psychotropic drugs such as the selective 5-HT reuptake inhibitor (SSRI) antidepressants or the anticonvulsant valproic acid (VPA). We perform a comparative analysis, identifying differences and commonalities in the behavioral effects produced in these models. Furthermore, this review discusses recent advances on neurodevelopmental substrates engaged in these behavioral effects, emphasizing the possible existence of maladaptive mechanisms that could be shared by the different models.

Introduction
Mental disorders including depression and anxiety are devastating and disabling conditions for the individual’s life, with an extremely concerning high prevalence worldwide. Currently, around 4.4% and 3.6% of the global population suffers from depressive and anxiety disorders, respectively, while the burden of mental illnesses is continuously rising1,2.

Psychiatric disorders arise from a complex combination of genetic, biological, environmental, and psychosocial factors. However, one of the main risk factors contributing to psychopathology corresponds to early-life adverse experiences during childhood, especially those involving traumatic or stressful events, such as maltreatment, abuse, poor or neglectful parenting, and loss of a family member, among others3–7. Furthermore, adverse experiences not only could contribute to pathological mental states, but may also affect the effectiveness of prospective therapeutic treatments7,8.

Brain imaging studies have shown that childhood maltreatment results in persistent alterations in a wide repertoire of neurocognitive systems involved in threat processing, reward, emotions, and executive control6,8,9. Interestingly, such alterations can often be found even in the absence of psychiatric conditions, indicating that maladaptive mechanisms produced by adverse environments in the early-life could predispose to latent vulnerabilities to future psychiatric disorders8,9.
Early-life exposure to certain drugs that interfere with the normal neurodevelopmental trajectories could also increase the predisposition of individuals to develop psychiatric disorders. In particular, chemical substances that can interfere with crucial molecular and cellular neurodevelopmental events (e.g., cell proliferation, migration, differentiation, axon growth, synaptic connectivity, etc...) during the so-called critical periods. These critical periods could represent unique developmental windows of brain plasticity, particularly relevant for preventive or therapeutic interventions.

One example of this, is the use of antidepressant drugs that act as selective serotonin (5-HT) reuptake inhibitors (SSRIs) in pregnant and lactating depressed mothers. These molecules have as a main molecular target the 5-HT transporter (SERT) typically present at the axon terminals of 5-HT neurons. Accumulating clinical evidence indicates that perinatal exposure to SSRIs could have a detrimental impact on infant’s neurodevelopment, with long-lasting consequences on cognitive and emotional capabilities.

In addition, other lines of evidence indicate that early exposure to SSRIs during pregnancy could increase the risk of Autism Spectrum Disorders (ASD), which in turn present a robust comorbidity with other psychiatric disorders including anxiety and depression. However, there is a general agreement that adverse consequences observed after antenatal SSRI treatments should be always contrasted against the highly detrimental consequences of the unmedicated maternal mental illness.

To investigate the impact of early-life adverse experiences and exposure to drugs that could interfere with the normal neurodevelopment, several preclinical rodent models were developed, in which many of the emotional, social and cognitive aspects of human psychopathology can be recapitulated. These models represent very useful tools to interrogate different aspects of the highly complex human pathophysiology, likely implying dysregulation of neurotransmitter systems, hormones and neuropeptides, growth factors, immune and inflammatory molecules, etc...

In addition, brain structures such as prefrontal and sensory cortical regions, as well as other limbic regions including the amygdala, hippocampus and brainstem nuclei, have been increasingly driving the attention of clinicians and researchers as plausible neurodevelopmental substrates contributing to the vulnerability to mental illness.

In this review, we explore well-established rodent models applied to study the impact of early-life environmental perturbations on adult behaviors of relevance to psychiatric disorders. We limited our analysis to models in which alterations in the 5-HT neurotransmission system appear to play a crucial role in the pathophysiological mechanisms. Thus, we perform a comparative analysis across models of early-life exposures to stress and psychotropic drugs such as SSRIs or valproic acid (VPA), identifying differences and commonalities in their emotional and social behavioral outcomes, but also considering the impact of the perinatal period of exposure and treatment schedule on these effects. Importantly, this review discusses recent advances on developmental neural substrates engaged in such behavioral outcomes, also identifying possible maladaptive mechanisms likely shared by these models. To this aim, we carry out an exhaustive search in the PubMed database until February 2021, using a combination of the following key words: SSRI, fluoxetine, citalopram, serotonin, stress, early-life adversity, maternal separation, restrain, limited bedding/nesting, valproate, VPA, prenatal, gestational, pregnancy, postnatal, lactation, critical period, rodent, rat, mouse, emotional behavior, anxiety, depression, and mood. The results of this search were further refined and narrowed down to obtain only those studies that had sufficient methodological details to allow comparative analyses across the different models, as well as studies investigating the possible neurodevelopmental mechanisms contributing to the emotional and social behavioral effects.

**Early-life stress models: behavioral outcomes**

In consonance with the primordial role of the early-life adverse experiences in the etiology of psychiatric disorders, several rodent models were established in which the dams and/or the offspring are exposed to different stressful stimuli during different neurodevelopmental stages. Importantly, in these models many of the emotional and cognitive impairments present in the human psychopathology can be consistently replicated.

**Gestational stress: restraint, unpredictable stress**

Two of the more frequently applied stressors to dams during the gestation are the restraint and unpredictable stress protocols. While the first one implies the physical body restraint of the dams in a closed compartment for time periods ranging from 1.5 to 6 h per day, in the second one, dams are daily subjected to a variety of different stressful stimuli including exposure to anxiogenic environments (social defeat paradigm), foot shocks (learned helplessness paradigm), forced swim, among others. Besides, these models vary according to whether the stress protocol is applied during the entire gestational period (i.e., until the offspring birth) or selectively during defined gestational windows, often resulting in distinctive behavioral outcomes.

Daily application of restraint or unpredictable stress protocols during almost the entire gestational period, in various rat and mouse strains, produces several stress-related and emotional alterations in the offspring later in life (Table 1). One of the most consistently reproduced
| Stress type                        | Exposure period | Rodent model                      | Behavioral phenotypes                                                                 | References                      |
|-----------------------------------|-----------------|-----------------------------------|---------------------------------------------------------------------------------------|--------------------------------|
| Restraint (6 h/day)               | G5.5–G17.5      | ICR mice                          | ▼ Rearing and Locomotion (OF)                                                        | Miyagawa et al.66,67             |
|                                   |                 |                                   | ▼ Time and Entries in open arms (EPM)                                                 |                                |
|                                   |                 |                                   | ◄ Rearing and Head dipping (Hole-board test)                                           |                                |
| Restraint (2.25 h/day)            | G7–P0           | Swiss albino ND4 mice              | ▼ Time and Entries in open arms (EPM)                                                 | Dong et al.68                    |
|                                   |                 |                                   | ◄ Time in dark compartment (Light-dark test)                                          |                                |
|                                   |                 |                                   | ◄ Alcohol consumption (2-bottle free choice)                                          |                                |
| Restraint (2.25 h/day)            | G12–G18         | C57BL/6NCr mice                   | ▼ Time in open arms (EPM)                                                              | Akatsu et al.55                  |
|                                   |                 |                                   | = No effects (MWM)                                                                   |                                |
| Restraint (2.25 h/day)            | G15–P0          | Swiss albino mice                 | ◄ Exploration and locomotion (T-maze, Tight-rope) (in males)                           | Pallarés et al.51                |
|                                   |                 |                                   | ◄ Entries in open arms (EPM) (in females)                                             |                                |
| Restraint (1.5 h/day)             | G5–G19          | Wistar and Lewis rats NMRI and C57BL/6 mice | = No effects (OF)                                                                     | Enayati et al.49                |
|                                   |                 |                                   | ◄ Sucrose preference (SPT) and ◄ Latency to feed (NSF)                                |                                |
|                                   |                 |                                   | ◄ Immobility time (TST, FST)                                                          |                                |
| Restraint (2.25 h/day)            | G14–G21         | CD rats                           | ◄ Entries in open arms (EPM) (in females)                                             | Richardson et al.50             |
|                                   |                 |                                   | ◄ Time in open arms (EPM) (in males)                                                 |                                |
| Restraint (2.25 h/day)            | G11–P0          | Sprague-Dawley rats               | ◄ Time in the center (OF)                                                             | Van den Hove et al.54           |
|                                   |                 |                                   | ◄ or ◄ Time in open arms (EPM) (in males or females)                                  |                                |
|                                   |                 |                                   | ◄ Spatial learning (MWM) (in females)                                                 |                                |
| Restraint (2.25 h/day)            | G14–G21         | Sprague-Dawley rats               | ◄ Time in the center (OF) (in males)                                                  | Iturra-Mena et al.57            |
|                                   |                 |                                   | ◄ Social interaction (in both sexes)                                                  | Poltyrev et al.58               |
|                                   |                 |                                   | ◄ Climbing and ◄ Immobility time (FST) (in males)                                      |                                |
|                                   |                 |                                   | = No effects (SPT)                                                                   | Soliani et al.56                |
|                                   |                 |                                   | ◄ Object recognition (in females)                                                     | Barbie-Shoshani et al.59        |
|                                   |                 |                                   | (depending on exposure period)                                                        |                                |
| Unpredictable chronic stress (1/day) | G1–G7          | Wistar rats                       | = or ◄ Avoidance (ETM)                                                               |                                |
|                                   |                 |                                   | (depending on exposure period)                                                        |                                |
| Unpredictable chronic stress (1/day) | G8–G14          |                                   | = No effects (SPT)                                                                   | Soliani et al.56                |
|                                   |                 |                                   | ◄ Object recognition (in females)                                                     |                                |
| Unpredictable chronic stress (80 foot shocks/day) | G0–P0          | Wistar rats                       | ◄ Time and entries in open arms (EPM)                                                 | Estanislau and Morato45         |
| Maternal separation (1 h/day)     | P1–P11          | C57BL/6 mice                      | ◄ Flexibility (4 choice-reversal learning)                                            | Thomas et al.83                 |
| Maternal and peer separation (4 h/day) | P2–P14          | C57BL/6 mice                      | = No effects (OF)                                                                    | Bailoo et al.70                 |
| Stress type               | Exposure period | Rodent model         | Behavioral phenotypes                                                                 | References |
|--------------------------|-----------------|----------------------|---------------------------------------------------------------------------------------|------------|
| Maternal separation      | P2–P14          | C57BL/6 mice         | ↓ Distance traveled and Rearings (in males) (OF)                                       | Bondar et al.65 |
| (4 h/day)                |                 |                      | ↓ Time in open arms (in females) (EPM)                                                 |            |
|                          |                 |                      | ↑ Social interactions (in females)                                                      |            |
| Maternal separation      | P2–P14          | C57BL/6 mice         | ↑ Time in the center and Rearings (OF)                                                 | Own and Patel69 |
| (3 h/day)                |                 |                      | ↓ Latency to first immobility (FST)                                                     | Teissier et al.67 |
|                          |                 |                      | ↓ Time in open arms (EPM)                                                                |            |
|                          |                 |                      | ↑ Marble burying                                                                       |            |
|                          |                 |                      | ↑ Immobility time (FST)                                                                 |            |
|                          |                 |                      | ↓ Grooming time (Splash test)                                                           |            |
|                          |                 |                      | ↓ Short-term memory (Sequential novel object recognition)                              |            |
| Maternal separation      | P2–P20          | C57BL/6 mice         | ↑ Time and Entries in closed arms (EPM)                                                 | Shin et al.79 |
| (4 h/day)                |                 |                      | = No effects (FST, Y-maze, MWM)                                                         |            |
|                          |                 |                      | ↑ Dominance (Tube test)                                                                  |            |
|                          |                 |                      | ↓ Latency to first attack and ↑ Number of attacks (Resident intruder test)              |            |
| Maternal separation      | P3–P21          | C57BL/6 mice         | ↑ Time in the center (OF) (after chronic social defeat stress)                         | Qin et al.72 |
| (1 h/day)                |                 |                      | ↑ Time and Entries in open arms (EPM) (after chronic social defeat stress)              |            |
| Maternal separation      | P10–P20         | C57BL/6J mice        | ↓ Time in the center (OF) (after stress defeat)                                         | Peña et al.64 |
| (4 h/day)                |                 |                      | ↑ Immobility time (FST) and ↓ Sucrose consumption (SPT) (after stress defeat)           |            |
|                          |                 |                      | ↓ Social interaction (after stress defeat)                                              |            |
| Maternal separation      | P2–P5           | C57BL/6J and DBA/2 mice | ↓ Time in the center (OF)                                                               | George et al.61 |
| (4 h/day)                |                 |                      | ↓ Entries in open arms (EPM)                                                            |            |
|                          | P6–P16          |                      | ↑ Immobility time (FST) (in DBA/2)                                                      |            |
|                          |                 |                      | + early weaning (at P17)                                                                |            |
| Maternal separation      | P7–P15          | C57BL/6J mice        | ↑ Immobility time (TST)                                                                 | Tchenio et al.82 |
| (6 h/day)                |                 |                      | ↓ Sucrose consumption (SPT)                                                             |            |
|                          |                 |                      | ↑ Failure to escable shocks (Shuttle box)                                               |            |
|                          |                 |                      | + early weaning (at P17)                                                                |            |
| Maternal separation      |                 |                      | ↓ Freezing to conspecific                                                              | Litvin et al.86 |
| Long Evans rats           |                 |                      |                                           |            |

Table 1 continued
| Stress type                        | Exposure period | Rodent model                     | Behavioral phenotypes                                      | References            |
|-----------------------------------|-----------------|----------------------------------|------------------------------------------------------------|-----------------------|
| (3 h/day)                         | P2–P13          | ↓ Unconditioned freezing (cat odor) |                                                           |                       |
| (6 h/day)                         | P11–P13         |                                  |                                                           |                       |
| Maternal separation (3 h/day)     | P1–P14          | Sprague-Dawley rats              | = No effects (OF)                                          | Farkas et al.68       |
| Maternal separation (3 h/day)     | P2–P14          | Wistar rats                      | ↓ Entries and Distance in the center (OF)                  | Benekareddy et al.62,63|
| Maternal separation (3 h/day)     | P2–P14          | Long Evans rats                  | ↑ Passive-submissive to proactive coping (Social Defeat)   | Gardner et al.85      |
| Maternal separation (3 h/day)     | P2–P15          | Wistar rats                      | ↓ Time in open arms (EPM)                                  | Uhelski and Fuchs76    |
| Maternal separation (1,3 h/day)   | P1–P14          | Wistar-Kyoto (WKY) and Wistar (W) rats | ↑ Exploration (in WKY) and ↓ (in W) (OF)                    | Rana et al.71         |
| Maternal separation (6 h/day)     | P2–P15          | Wistar rats                      | ↓ Latency to first immobility and ↑ Immobility time (FST)  | Roque et al.77        |
| Maternal and peer separation (6 h/day) | P4–P14          | Wistar rats                      | ↓ Latency to enter the dark and ↑ Time in the dark (Light-dark test) | Kambali et al.78      |
| Limited bedding and nesting material | P2–P9           | CS7BL/6J mice                    | = No effects (OF) and ↑ Latency to escape (MWM)           | Rice et al.73         |
| Limited bedding and nesting material | P2–P9           | CS7BL/6 mice                     | ↓ Novel object exploration                                 | Yang et al.74         |
| Limited bedding and nesting material | P2–P9           | CS7BL/6J mice                    | = No effects (OF) and ↓ Time and Entries in bright compartment (Light-dark test) | Naninck et al.81      |
Table 1 continued

| Stress type                        | Exposure period | Rodent model       | Behavioral phenotypes                          | References |
|------------------------------------|-----------------|--------------------|------------------------------------------------|------------|
| Limited bedding and nesting material | P4–P11          | C57BL/6 mice       | † Spatial learning (MWM) (in males)              | Gallo et al. |
|                                    |                 |                    | † Distance traveled (OF) (in kicked pups)       |            |
|                                    |                 |                    | Time in bright compartment (↑ in kicked and ↓ in non-kicked pups) (Light-dark test) |            |
|                                    |                 |                    | = No effects (O-maze)                            |            |

In the studies where both sexes were analyzed, the sex-specific effects observed are indicated.

OF: Open field, EPM: Elevated plus maze, EZM: Elevated Z-maze, MWM: Morris water maze, NSF: Novelty-suppressed feeding test, TST: Tail suspension test, FST: Forced-swim test, SPT: Sucrose preference test, ETM: Elevated T-maze.

Phenotypes observed in these models, is the enhancement of anxiety behaviors, often accompanied by reduced locomotor and exploratory activities45-49. Additionally, these anxiety effects can predispose the offspring to other compulsive addictive behaviors like alcohol consumption48 (Table 1). Importantly, stress exposure in the late phase of the pregnancy, somewhere within the period from gestational day (G) 11 to postnatal day (P) 0, in mice and rats, has also produced robust anxiety phenotypes50-57 (Table 1).

Depressive-like symptoms were also reported after gestational exposure to stress in rats49,58, though the evidence is more limited (Table 1).

Examination of other behavioral components associated with psychiatric conditions, such as the social interaction to conspecifics, showed a marked reduction after prenatal stress59. In contrast, cognitive functions do not seem to be substantially affected in these models59, though moderate improvements in spatial learning52,59 and object recognition memory59, were observed in the offspring (Table 1).

Postnatal stress: maternal separation, limited bedding and nesting material

Other models directly expose the offspring to the stressful stimuli. The repeated maternal separation of the pups from the dams has been consistently used as an efficient stressful condition, especially when it happens during the lactation period60. This model sometimes also includes other stressors like the early weaning of the pups or a limited access to the bedding and nesting materials.

Protocols of maternal separation vary according to the time the pups spend isolated from their dams, but also to during which postnatal period the protocol is applied. Thus, while the time of separation typically ranges from 1 to 8 h per day, the postnatal period usually covers the first 2 or 3 postnatal weeks. In addition, in these models, distinctive behavioral effects can be observed when considering the mouse/rat strain and the sex of the offspring.

Daily separation from the dams during the first 2 or 3 postnatal weeks, in various mouse/rat strains, has consistently produced a reduction in the exploratory activity of the offspring61-67. Besides, these effects appeared to be more commonly detected in males than in female littersmates65. However, other studies using slightly different conditions, could not reproduce these findings68-72. A combination of maternal separation with an early weaning of the pups also produced a decreased exploration61. On the other hand, stress protocols of limited access to bedding and nesting material, were shown to be inefficient per se to reproduce the exploratory defects73-75 (Table 1).

Anxiety phenotypes were consistently observed in various mouse/rat strains after maternal separation during the first 26,62,63,65,67,71,76-78 or 3 postnatal weeks63,64,66,79,80 (Table 1). However, milder protocols (1 h/day) appeared to be insufficient to produce anxiety effects, after a chronic social distress72. On the other hand, anxiety phenotypes were reported to be more evident in females than in male descendants65. Lastly, less robust anxiety phenotypes were observed when both the dams and pups had a limited access to the bedding and nesting material during early postnatal life72,73,75,81 (Table 1).

Depressive-like behaviors have been repeatedly observed after maternal separation protocols in various mouse strains64,67,69, though others failed to reproduce these effects79. Similar depressive-like symptoms were reported when the maternal separation was followed by an early weaning in mice61,82. Interestingly, a mouse study suggested that the maternal separation from P10 to P20 would be sufficient to induce depressive-like symptoms in the offspring64. In rats, depressive-like phenotypes produced by maternal separation protocols are less robust. Thus, depressive-like symptoms were reported in Sprague-Dawley and Wistar rats66,77, while other studies using briefer protocols (1.2 h/day) in Wistar-Kyoto rats, have described the opposite effects71 (Table 1).

The impact of early maternal separation upon cognitive behaviors was consistently evidenced (Table 1).
general, postnatal exposures to stress appeared to have more profound consequences on cognitive abilities than prenatal exposures. Studies carried out in various mouse strains have shown deficits in behavioral flexibility and short-term memory, after different maternal separation protocols. In contrast, no apparent effects were observed on spatial learning and working memory in mice. However, in rats, improvements in spatial learning and attention were recently described. Detrimental consequences of the limited bedding and nesting material have been observed on the novel object exploration and location memory, accompanied by deficits in spatial learning and working memory tasks (Table 1).

Consequences of maternal separation on social behaviors were reported in mice and rats (Table 1). Thus, a delayed latency to initiate social contacts, and a decreased engagement in social interactions and activities, have been found. However, other studies could not reproduce some of these effects. Interestingly, distinct behavioral features associated with social behaviors, like aggression or dominance, were also found to be enhanced by maternal separation. Consistent with this, a reduction of freezing responses to conspecifics, or after a punishment, were also observed (Table 1).

Pharmacological models: behavioral outcomes

Psychotropic drugs can interfere directly or indirectly with the brain’s developmental trajectory. Importantly, the age period when such perturbations occur will define the emergence of long-lasting detrimental consequences on the individual’s brain architecture and function. Rodent models have been very useful to understand how such dysregulation of neural mechanisms during developmental critical periods can impact adult behaviors. Here, we analyze the behavioral outcomes of two pharmacological models: the exposure to SSRI antidepressants, and to the anticonvulsant and mood stabilizer, VPA. Both drugs have a high capacity of crossing the placenta to reach the fetus, after different maternal separation protocols. In contrast, no apparent effects were observed on spatial learning and working memory in mice. However, in rats, improvements in spatial learning and attention were recently described. Detrimental consequences of the limited bedding and nesting material have been observed on the novel object exploration and location memory, accompanied by deficits in spatial learning and working memory tasks (Table 1).

Postnatal exposure to SSRIs

Other studies investigated the behavioral consequences of exposures to SSRIs during the early postnatal period. Fluoxetine application during the first 2 or 3 postnatal weeks in various mouse/rat strains resulted in a marked reduction of exploratory behaviors, accompanied by the emergence of anxiety and depressive-like phenotypes. Others, applying similar protocols of fluoxetine exposure, only partially reproduced these emotional effects. Postnatal exposure to other SSRIs like citalopram or escitalopram, during the same postnatal period, also produces anxiety and depressive-like phenotypes in mice. However, other studies have only partially replicated some of these emotional effects. In addition, citalopram exposure in the same period, was shown to reduce the exploration of novel objects and the...
| SSRI (daily dose) | Exposure period | Rodent model | Behavioral phenotypes | References |
|-------------------|----------------|--------------|-----------------------|------------|
| Fluoxetine (0.3–0.8 mg/kg i.p.) | G8–G18 | C57BL/6J mice | ↓ Distance traveled in the center (OF) † Time in closed arms (EPM) † Latency to feed (NSF) | Noorlander et al.92, Smit-Rigter et al.93 |
| Fluoxetine (10 mg/kg s.c.) | G1–P0 | CD1 mice | = No effects (Novel object exploration, Object memory test) † Animal exploration (Social preference test) (only in young females) = No effects (Social exploration and Social Memory tests) † Number and Duration of attacks (Social exploration and memory tests) | Svirsky et al.100 |
| Fluoxetine (25 mg/kg per os.) | G15–P12 | C57BL/6 mice | = No effects (OF) † Time in open arms and Number of head dips (EPM) † Spatial memory (MWM) = No effects (Passive avoidance, PPI) † Proportion of attackers = No effects (Social interaction) (Resident intruder) | Kiryanova et al.95,96 |
| Fluoxetine (25 mg/kg per os.) | G15–P12 | C57BL/6 mice | = No effects (OF, Horizontal ladder, PPI, MWM, Fear conditioning) † Time in closed arms (EPM) † Latency to first immobility (FST) = No effects (Social interaction) (Resident intruder) | McAllister et al.94 |
| Fluoxetine (10 mg/kg per os.) | G0–P14 | Sprague-Dawley and Wistar-Kyoto rats | ↓ Time in the center (OF) ❌ Time in open arms (EPM) † Immobility time (FST) | Millard et al.99 |
| Fluoxetine (10 mg/kg per gavage) | G0–P21 | Wistar rats | ↓ Third-party prosocial behavior (in females) | Heinla et al.102 |
| Fluoxetine (5 mg/kg per gavage) | G1–P21 | Wistar rats | = No effects (OF, EPM) | Toffoli et al.98 |
| Fluoxetine (10 mg/kg per os.) | G10–P21 | Sprague-Dawley rats | in females: † Time interacting with another female † Time in social investigation † Time to first interaction in males: † Time in social play † Running away from a novel partner † Self grooming | Gemmel et al.101 |
| SSRI (daily dose)                     | Exposure period | Rodent model                  | Behavioral phenotypes                                                                 | References       |
|--------------------------------------|-----------------|-------------------------------|--------------------------------------------------------------------------------------|------------------|
| Fluoxetine (12 mg/kg per gavage)     | G11–P0          | Wistar rats                   | No effects (OF, EPM, SPT, FST)                                                       | Olivier et al.  |
|                                      |                 |                               | ↑ Latency to feed (NSF)                                                               | 97               |
|                                      |                 |                               | ↑ Freezing and ↓ Time in the shock compartment                                         |                  |
|                                      |                 |                               | (Place aversion)                                                                     |                  |
|                                      |                 |                               | ↓ Juvenile social play and exploration                                               |                  |
|                                      |                 |                               | ↓ Adult self-grooming and social exploration                                         |                  |
| Fluoxetine (12 mg/kg per gavage)     | G11–P7          | Wistar rats                   | ↑ Turning time (Negative geotaxis)                                                    | Kroeze et al.   |
|                                      |                 |                               | ↑ Age (Vibrissa placement)                                                            | 61               |
|                                      |                 |                               | ↑ Age (Startle reflex)                                                                |                  |
|                                      |                 |                               | ↓ Locomotor and motor abilities (at early postnatal ages)                            |                  |
|                                      |                 |                               | ↓ Grooming                                                                           |                  |
|                                      |                 |                               | = No effects (NOR, Object directed behavior)                                           |                  |
| Fluoxetine (10 mg/kg i.p.)           | P4–P21          | 129S6/SlcEvTac mice           | ↓ Distance traveled, Rearing and Ambulation times (OF)                               | Ansorge et al.  |
|                                      |                 |                               | ↓ Number of open arm entries (EPM)                                                    | 103              |
|                                      |                 |                               | ↑ Latency to escape (shock-avoidance/escape)                                          |                  |
|                                      |                 |                               | ↑ Latency to feed (NSF)                                                               |                  |
| Fluoxetine (10 mg/kg i.p.)           | P2–P11          | 129S6/SlcEvTac mice           | ↓ Sucrose consumption (SPT)                                                           | Rebello et al.  |
|                                      |                 |                               |                                                                                     | 106, Teissier et al. |
| Fluoxetine (10 mg/kg per os.)        | P2–P14          | C57BL/6J mice                 | ↑ Immobility time (FST)                                                              | Soiza-Reilly et al. |
|                                      |                 |                               |                                                                                     | 111              |
| Fluoxetine (10 mg/kg s.c.)           | P2–P14          | C57BL/6J mice                 | ↑ Immobility time (FST)                                                              | Olusakin et al. |
|                                      |                 |                               |                                                                                     | 112              |
|                                      |                 |                               | ↑ Time and Total distance in the center (OF)                                          |                  |
|                                      |                 |                               | ↑ Latency to feed (NSF)                                                               |                  |
|                                      |                 |                               | ↑ Immobility time (FST)                                                              |                  |
|                                      |                 |                               | ↑ Latency to groom (Splash test)                                                      |                  |
| Fluoxetine (5 mg/kg per os.)         | P1–P21          | C57BL/6J mice                 | No effects (MWM)                                                                     | Ishiwata et al. |
|                                      |                 |                               |                                                                                     | 119              |
| Fluoxetine (10 mg/kg i.p.)           | P4–P21          | C57BL/6J mice                 | Immobility time in the center, Total rearing time (OF)                               | Karpova et al.  |
|                                      |                 |                               |                                                                                     | 113              |
|                                      |                 |                               | Total immobility time (Light-Dark)                                                   |                  |
|                                      |                 |                               | ↓ Immobility time (FST)                                                              |                  |
| Fluoxetine (5 mg/kg per os.)         | P1–P21          | BALB/c mice                   | ↑ Time and Entries in open arms (EPM)                                                 | Ishikawa and Shiga |
|                                      |                 |                               | = No effects (FST, SPT, MWM)                                                         | 116              |
| SSRI (daily dose) | Exposure period | Rodent model       | Behavioral phenotypes                                                                 | References         |
|-------------------|-----------------|--------------------|----------------------------------------------------------------------------------------|--------------------|
| Fluoxetine (10 mg/kg s.c.) | P0–P6          | Wistar rats        | ↓ Maximum crossable gap distance (Gap-crossing) <br>↓ Ambulation in the center and rearing (OF) | Lee et al.117       |
| Fluoxetine (20 mg/kg s.c.) | P0–P4          | Wistar rats        | ↓ Distance traveled and ambulation in the center (OF) <br>↓ Number of closed arm entries and of total distance traveled (EPM) <br>↑ Time spent in immobility (FST) <br>↓ Sensorimotor gating (PPI) <br>↑ Social interaction, sniffing and contacts | Ko et al.115       |
| Fluoxetine (10 mg/kg per gavage) | P2–P7         | NIH Norway rats    | ↓ Ultrasonic vocalizations (PPI) <br>↓ Interaction time with conspecific | Zimmerberg and Germeyan118 |
| Fluoxetine (5 mg/kg s.c. osmotic minipump in dams) | P1–P21        | Sprague-Dawley rats | in females: <br>↓ No effects (OF, EZM) <br>↑ Immobility time (FST) <br>↓ Center entries (OF) <br>↓ Distance traveled (EZM) <br>↓ No effects (FST) | Boulle et al.100,110 |
| Fluoxetine (10 mg/kg s.c.) | P1–P21         | Wistar rats        | ↓ Time in closed arms <br>↑ Number of open arm entries and Time in open arms (EPM) | Da Silva et al.114 |
| Fluoxetine (10 mg/kg per os.) | P2–P21         | Sprague-Dawley rats | ↓ Time and Traveled distance in the center (OF) <br>↓ Path length and time in open arms (EPM) <br>↑ Immobility time (FST) <br>↓ Juvenile play behavior and Time in social grooming | Sarkar et al.104,105 |
| Fluoxetine (10 mg/kg i.p. in dams) | P2–P24           | Sprague-Dawley rats | ↓ Time in closed arms (EPM) (in males) <br>↑ Latency to feed (NSF) (in males) <br>↑ Swim time (FST) | Gobinath et al.108 |
| Citalopram (10 mg/kg i.p.) | P4–P21         | 12956/SvEv mice    | ↓ Total ambulatory time (OF) <br>↓ Total number of arm entries (EPM) <br>↑ Latency to escape (shock-escape) <br>↑ Latency to drink (novelty-induced hypophagia) | Ansorge et al.121 |
| Citalopram (20 mg/kg s.c.) | P1–P10         | Sprague-Dawley rats | ↓ Total ambulatory time (OF) <br>↓ Total number of arm entries (EPM) <br>↑ Latency to escape (shock-escape) <br>↑ Latency to drink (novelty-induced hypophagia) | Zhou et al.214     |
engagement in juvenile play, besides of exacerbating the freezing response to a tone\textsuperscript{126–128} (Table 2).

Since SSRIs are often prescribed to depressed pregnant women, preclinical investigations on the possible interaction of these treatments with the maternal stress become highly relevant for obvious direct translational reasons. Several studies have shown that early postnatal fluoxetine treatment in pups exposed to either prenatal stress or maternal separation, can alleviate anxiety and depressive-like symptoms produced in these models\textsuperscript{110,129–131}. However, this does not seem to be the case when using another SSRI (i.e., citalopram)\textsuperscript{132}.

### Gestational and postnatal exposures to VPA

Acute treatment with a high dose of VPA during pregnancy causes a wide repertoire of emotional, social and cognitive alterations in the offspring (Table 3). Although the exact molecular mechanism of action of this drug remains unknown, VPA has been consistently applied during neurodevelopment in an attempt to replicate common phenotypic features present in ASD patients\textsuperscript{39,40}.

Most of the studies reporting behavioral effects in various mouse/rat strains use a single dose of VPA (400–800 mg/kg) in a given day, within the period G11–G13 (Table 3). In these conditions, the exposed-offspring presents substantial neurological maturation delays\textsuperscript{133–135}, usually accompanied by a reduced exploratory activity\textsuperscript{136–138}, and the enhancement of self-grooming and stereotypic behaviors\textsuperscript{133–136,138–148}. This is highly consistent with the exacerbation of repetitive behaviors observed in ASD patients\textsuperscript{39,40}. Interestingly, long-lasting deficits in motor and procedural skills were also observed after VPA exposure\textsuperscript{144}. On the contrary, neither lower doses nor VPA exposures before or after such gestational period, lead to robust behavioral outcomes\textsuperscript{133,136,137,149–151} (Table 3).

Anxiety phenotypes induced by VPA exposure during the pregnancy were more consistently found in rats than in mice\textsuperscript{135,137–139,142,146,147,152–157}. However, other studies could not replicate some of these effects\textsuperscript{144}. On the other hand, the emergence of depressive-like symptoms was also reported in a mouse study\textsuperscript{147} (Table 3).
| VPA dose       | Exposure period | Rodent model                  | Behavioral phenotypes                                                                 | References                                      |
|---------------|-----------------|-------------------------------|----------------------------------------------------------------------------------------|------------------------------------------------|
| 500 mg/kg i.p. | G9, G12.5, or G14.5 | ICR(CD1) mice               | ↓ Distance and Entries in the center (OF) (for G12.5, in both sexes)                   | Kataoka et al. [137]                           |
|               |                 |                               | ↓ Time in open arms (EPM) (for G12.5, in both sexes)                                   |                                                 |
|               |                 |                               | ↓ Social interaction (Sniffing) (for G12.5, in males)                                  |                                                 |
|               |                 |                               | ↑ Social interaction (Sniffing) (for G12.5, in females)                                 |                                                 |
|               |                 |                               | ↓ Spatial learning (MWM) (for G12.5)                                                   |                                                 |
| 800 mg/kg per os. | G11            | Hybrid mice (C57BL/6, CF-1, Swiss, DBA/2) | ↑ Latency to reach home bedding                                                       | Roullet et al. [158]                           |
|               |                 |                               | ↓ Social behavior (Nose pokes)                                                         |                                                 |
|               |                 |                               | ↓ Social novelty (Nose pokes)                                                          |                                                 |
|               |                 |                               | = No major effects (Negative geotaxis, Surface righting, Balance beam).                 | Wagner et al. [133]                            |
|               |                 |                               | ↑ Delay (Surface and Mid-air righting) (in both sexes)                                 |                                                 |
| 200 mg/kg s.c. | G12–G17         | BALB/c mice                  | ↓ Latency to fall (Grip strength) (in both sexes)                                      |                                                 |
| 600 mg/kg s.c. | G13             |                               | ↑ Locomotor activity                                                                   |                                                 |
| 600 mg/kg s.c. | G12.5           | Crl:Fcen:CF1 mice            | ↓ Juvenile play (Solicitations, sniffing, Following)                                   |                                                 |
|               |                 |                               | ↑ Self-grooming                                                                        |                                                 |
|               |                 |                               | ↓ Alternation and distance (Y-maze, EPM, OF)                                          |                                                 |
|               |                 |                               | = No effects (Affiliative and Non-social behaviors; Odor habituation, NOR, Light-     |                                                 |
|               |                 |                               | dark test                                                                              |                                                 |
|               |                 |                               | ↓ Social preference and interaction (Sniffing) (in males)                               |                                                 |
|               |                 |                               | ↑ Immobility time (TST, FST)                                                           |                                                 |
|               |                 |                               | ↓ Temporal Accuracy and Precision (Interval timing)                                    |                                                 |
| 500 mg/kg i.p. | G12.5           | C57BL/6J mice                | ↑ Marble burying                                                                       | Wu et al. [164]                                |
|               |                 |                               | ↓ Social interaction (time)                                                            |                                                 |
|               |                 |                               | ↓ Social preference (time)                                                             |                                                 |
|               |                 |                               | ↓ Learning (Negative Reinforcement Task)                                               |                                                 |
| 600 mg/kg i.p. | G12.5           | C57BL/6J mice                | ↑ Grooming and Digging time                                                            | Moldrich et al. [145]                          |
|               |                 |                               | ↓ Social interaction (time, nose pokes, approaches)                                    |                                                 |
| 600 mg/kg s.c. | G13             | C57BL/6Hsd mice              | ↓ Olfactory motivation                                                                | Mehta et al. [138]                             |
|               |                 |                               | ↑ Entries and Time in the center (OF)                                                   |                                                 |
|               |                 |                               | ↑ Self-grooming and Marble burying                                                     |                                                 |
| 800 mg/kg per os. | G9             | Wistar rats                  | ↑ Time of all pellet consumption and Exploration (Radial maze)                         | Nanta et al. [136]                             |
|               |                 |                               | ↑ Locomotor activity without anxiety effects (OF)                                      |                                                 |
|               |                 |                               | = No effects (Social interaction)                                                       |                                                 |
| 600 mg/kg i.p. | G9              | Wistar rats                  | ↑ Time spent in empty compartment, ↑ Crossings between social compartments, ↓          | Dufour-Rainfray et al. [149]                   |
|               |                 |                               | Initiation of social approaches                                                       |                                                 |
| 800 mg/kg per gavage | G9        | Wistar rats                  | ↑ Locomotor activity (OF) (in light/sleep phase)                                       | Tsujiuno et al. [141]                          |
|               |                 |                               | ↑ Feeding (in light/sleep phase)                                                        |                                                 |
| 500 mg/kg i.p. | G11.5           | Wistar Han rats              | ↑ Entries to the same arm (Y-maze)                                                     | Favre et al. [130]                             |
|               |                 |                               | ↓ Social preference (sniffing)                                                          |                                                 |
| 600 mg/kg i.p. | G12.5           | Wistar rats                  | ↑ Freezing (Pavlovian fear conditioning)                                               |                                                 |
|               |                 |                               | ↑ Latency to reach home bedding (olfactory discrimination in pups)                     |                                                 |
|               |                 |                               | ↓ Angle of swim (in pups)                                                              |                                                 |
| VPA dose      | Exposure period | Rodent model                  | Behavioral phenotypes                                                                 | References                  |
|--------------|----------------|------------------------------|---------------------------------------------------------------------------------------|-----------------------------|
| 500 mg/kg i.p.| G12.5          | Wistar Han rats              | ↑ Locomotor and Stereotypic behavior (in both sexes)                                    | Markram et al.\textsuperscript{142} |
|              |                |                              | ↓ Exploratory activity (Rearing and hole poking)                                        |                             |
|              |                |                              | ↓ Entries and Time in open arms (EPM) (in males)                                        |                             |
|              |                |                              | ↓ Sensorimotor gating (PPI)                                                             |                             |
|              |                |                              | ↓ Social play and Social exploration behavior (in males)                                 |                             |
|              |                |                              | = No effects (NOR)                                                                     |                             |
| 500 mg/kg i.p.| G12.5          | Wistar rats                  | ↑ Entries to the same arm (Y-maze)                                                     | Edalatmanesh et al.\textsuperscript{146} |
|              |                |                              | ↓ Time in open arms (EPM)                                                               |                             |
|              |                |                              | ↓ Social interaction (sniffing, touching)                                               |                             |
|              |                |                              | ↓ Sensorimotor gating (PPI)                                                             |                             |
|              |                |                              | ↑ Tone and Context memories, Generalization and Extinction (Fear conditioning)           |                             |
|              |                |                              | = No effects (Locomotion, MWM)                                                         |                             |
| 400 mg/kg s.c.| G12.5          | Wistar rats                  | ↓ Time in open arms (EPM)                                                               | Ellenbroek et al.\textsuperscript{155} |
|              |                |                              | ↑ Latency to feed (NSF)                                                                |                             |
|              |                |                              | ↑ Sucrose consumption (Latent inhibition)                                               |                             |
|              |                |                              | ↓ Sensorimotor gating (PPI)                                                             |                             |
| 600 mg/kg i.p.| G12.5          | Wistar rats                  | ↓ Social exploration and preference                                                      | Bambini-Junior et al.\textsuperscript{143} |
|              |                |                              | ↑ Altention delay (Y-maze)                                                              |                             |
|              |                |                              | = No effects (MWM)                                                                     |                             |
| 600 mg/kg i.p.| G12.5          | Wistar rats                  | ↓ Time in the center (OF) (in both sexes)                                               | Olesová et al.\textsuperscript{156} |
| 400 mg/kg s.c.| G7, G9.5, G12 or G15| Sprague-Dawley rats          | ↓ Interaction to familiar and novel conspecifics (more robust for G12 exposure, in males)| Kim et al.\textsuperscript{158,159} |
| 500 mg/kg per gavage | G11–13 | Sprague-Dawley rats          | ↓ Ultrasound vocalizations (in both sexes)                                               | Barrett et al.\textsuperscript{157} |
|              |                |                              | ↓ Time in the center (OF) (in females)                                                   |                             |
|              |                |                              | ↑ Baseline startle amplitude (in males)                                                   |                             |
|              |                |                              | ↑ Startle response (after Fear conditioning) (in both sexes)                             |                             |
|              |                |                              | ↓ Approach to a social stimulus (in both sexes)                                         |                             |
|              |                |                              | ↓ Novel social interaction (in males)                                                     |                             |
| 600 mg/kg i.p.| G12            | Sprague-Dawley rats          | ↑ Freezing (Trace and Delay Fear conditioning)                                          | Sui and Chen\textsuperscript{165} |
| 500 mg/kg i.p.| G12.5          | Sprague-Dawley rats          | ↑ Time in the center (OF)                                                               | Lin et al.\textsuperscript{153} |
|              |                |                              | ↓ Time in open arms (EPM)                                                               |                             |
|              |                |                              | ↓ Social interaction (duration and frequency)                                            |                             |
|              |                |                              | ↑ Freezing (Contextual fear conditioning)                                               |                             |
| 500 mg/kg i.p.| G12.5          | Sprague-Dawley rats          | ↓ Ultrasound vocalizations (in both sexes)                                               | Gzielo et al.\textsuperscript{163} |
| 600 mg/kg i.p.| G12.5          | Sprague-Dawley rats          | ↑ Delays in Surface and Air righting reflexes, Negative geotaxis, Cliff aversion, Crawling and Visual placing reflex | Hou et al.\textsuperscript{135} |
|              |                |                              | ↓ Motor abilities (Swimming, Front limb suspension)                                     |                             |
|              |                |                              | ↑ Onset of auditory startle                                                             |                             |
|              |                |                              | ↑ Self-grooming                                                                        |                             |
|              |                |                              | ↓ Center entries (OF)                                                                  |                             |
|              |                |                              | ↓ Social preference and interaction                                                     |                             |
|              |                |                              | ↓ Novel social interaction                                                             |                             |
|              |                |                              | ↓ Spatial learning (MWM)                                                                |                             |
Social behaviors appeared to be markedly affected by prenatal VPA in various mouse/rat strains, indicating a highly consistent reduction in the number of ultrasonic vocalizations and social interactions (e.g., nose pokes, approaches, etc...), accompanied by a diminished juvenile social play, and limited interest for novel conspecifics. However, several studies failed to replicate some of these

### Table 3 continued

| VPA dose                  | Exposure period | Rodent model          | Behavioral phenotypes                                                                 | References                  |
|---------------------------|-----------------|-----------------------|---------------------------------------------------------------------------------------|------------------------------|
| 500–600 mg/kg i.p.        | G12.5           | Sprague-Dawley rats   | ↑ Freezing (Fear conditioning)                                                        | Wang et al.166, Banerjee et al.154 |
|                           |                 |                       | ↓ Entries and Distance traveled in the center (OF)                                    |                              |
|                           |                 |                       | ↓ Social interaction and Social visits                                                 |                              |
|                           |                 |                       | = No effects (NOR)                                                                    |                              |
| 800 mg/kg per os.         | G12             | Long Evans rats       | ↓ Performance (T-maze) (in both sexes)                                                 | Mychasiuk et al.144          |
|                           |                 |                       | ↑ Time in open arms (EPM) (in both sexes)                                               |                              |
|                           |                 |                       | ↑ Interaction to familiar object (NOR) (in both sexes)                                  |                              |
|                           |                 |                       | ↑ Performance (Whishaw tray reaching test) (in males)                                    |                              |
|                           |                 |                       | ↓ Performance (Whishaw tray reaching test) (in females)                                  |                              |
| 600 mg/kg i.p.            | G12             | Long Evans rats       | ↓ Sniffing (in females)                                                               | McKinnell et al.148          |
|                           |                 |                       | ↑ Self-grooming (in both sexes)                                                        |                              |
|                           |                 |                       | ↑ Interaction to familiar object (NOR) (in males)                                       |                              |
|                           |                 |                       | ↓ Marble burying                                                                       |                              |
|                           |                 |                       | ↓ Performance (Set shifting task) (in both sexes, stronger effects in females)          |                              |
| 800 mg/kg per os.         | G12.5           | Long Evans rats (females) | ↓ Defensive rotation tactic and ↑ Standing tactic                                    | Raza et al.161               |
|                           |                 |                       | ↑ Frequency of Mounting, Head and Body shaking                                      |                              |
|                           |                 |                       | ↓ Ultrasonic vocalizations (at Social play)                                             |                              |
|                           |                 |                       | = No effects (Playful attacks)                                                        |                              |
| 350 mg/kg i.p.            | G13             | Long Evans rats       | ↑ Social exploration and Play fighting (in adolescence)                                | Cohen et al.151              |
| 200–400 mg/kg s.c.        | P14             | BALB/c mice           | = No effects (Grip strength, Balance beam, Locomotor activity) and ↑ Delay in Negative geotaxis and Mid-air righting | Wagner et al.143             |
|                           |                 |                       | ↓ Spatial learning (MWM) and ↑ Latency (Passive avoidance)                            |                              |
| 400 mg/kg s.c.            | P14             | BALB/c mice           | ↓ Social behaviors (allogrooming, crawl under/over, sniffing)                          | Yochum et al.170             |
|                           |                 |                       | ↓ Motor activity (in social environment)                                               |                              |
|                           |                 |                       | ↑ Locomotor activity                                                                   |                              |
| 400 mg/kg s.c.            | P14             | C57BL/6J mice         | ↑ Time in open arms (EPM) (only in males)                                              | Norton et al.169             |
|                           |                 |                       | ↓ Reversal learning (Water Y-maze)                                                     |                              |
|                           |                 |                       | ↑ Social aggressions (only in males)                                                    |                              |
|                           |                 |                       | = No effects (Locomotion, Social approach, PPI, Allogrooming, Sniffing)                |                              |
| 300 mg/kg s.c.            | P2–P4           | Sprague-Dawley rats   | ↑ Exploration (OF) and ↓ Entries and Time in open arms (EPM)                          | Mony et al.171               |
| (twice/day on P2–P3 and once at P4) |               |                       |                                                                                       |                              |
|                           |                 |                       | ↓ Social preference and interaction (Time spent, Sniffing, Grooming, Mounting, Crawling) |                              |
|                           |                 |                       | = No effects (Passive avoidance)                                                       |                              |
| 150 mg/kg/day i.p.        | P6–P20          | Sprague-Dawley rats   | ↑ Delay in eye opening                                                                | Chomiak et al.158            |
|                           |                 |                       | ↓ Social play (rough-and-tumble)                                                       |                              |
|                           |                 |                       | ↑ Cue-dependent reward learning                                                        |                              |

In the studies where both sexes were analyzed, the sex-specific effects observed are indicated.

OF Open field, EPM Elevated plus maze, PPI Prepulse inhibition, MWM Morris water maze, NSF Novelty-suppressed feeding test, TST Tail suspension test, FST Forced-swim test, NOR Novel object recognition.
Thus, depending on the period when the environmental stress occurs, a decreased olfactory motivation and sensorimotor capacity were found in VPA-exposed mice\cite{136,137,151}, (Table 3). Additionally, sex-specific aggressive and defensive strategies in social settings appeared to be affected by the VPA exposure, switching from a defensive tactic to a more aggressive one\cite{161}. Furthermore, enhanced freezing and startle responses were described in different fear-conditioning paradigms after VPA treatment\cite{142,153,154,157,160,165,166} (Table 3).

Certain cognitive aspects have been described to be altered in VPA gestational models, though the behavioral outcomes were less consistent. Thus, several studies reported either deficits\cite{133,135,137} or improvements in spatial learning\cite{146}, while others could not reproduce these effects\cite{142,143}. Similarly, evaluation of exploration time of a novel object showed that VPA treatment increased the interaction to familiar objects\cite{144,148}, though other researchers could not replicate these findings\cite{147,152,154}. Additionally, deficits in working memory\cite{146,147}, temporal accuracy\cite{167}, attentional, and negative reinforcement learning tasks\cite{148,164} were also reported (Table 3).

There are fewer studies applying postnatal exposures to VPA, having in general, less robust behavioral effects (Table 3). VPA treatment somewhere during the first 2 postnatal weeks produces a delayed neurological maturation\cite{133,168}, without any apparent locomotor effects\cite{133,169}, though others observed an enhanced locomotion\cite{170,171}. On the other hand, changes on anxiety behaviors were described in rats\cite{171}, but not in mice\cite{169} (Table 3).

Social behaviors were shown to be affected by postnatal exposure to VPA in rats and mice\cite{168,170,171}, though others could not replicate some of these findings\cite{169}. In the same conditions, higher levels of social aggression were found\cite{169} (Table 3).

Several cognitive aspects appeared to be affected in these models, including deficits in spatial\cite{133} and reversal learning\cite{169}, accompanied by enhanced cue-dependent reward learning\cite{168}. Additionally, other studies described longer latencies in a passive avoidance paradigm in mice\cite{133}, while others failed to reproduce some of these effects in rats\cite{171} (Table 3).

**Neural mechanisms engaged in the rodent models of early-life exposure**

In the last decade, many preclinical studies have contributed to our understanding of the possible molecular, cellular and circuit mechanisms implicated in the neurodevelopmental vulnerability to psychiatric conditions. A main concept emerging from those studies is that the ontogenetic occurrence of unique developmental events defines critical periods of plasticity with a maximal sensitivity to environmental functional demands\cite{14–16,18}. Thus, depending on the period when the environmental challenge/perturbation takes place, the long-lasting impact that it will have on the mature brain. In this section, we explore maladaptive neural mechanisms that have been implicated in the behavioral outcomes of the analyzed rodent models, paying special attention to brain regions and neural mechanisms that could be similarly altered across the different models.

**Developmental role of 5-HT and Prefrontal circuits**

Perturbation of the 5-HT neural signaling during early-life has been long associated with developmental origins of several psychiatric conditions, including anxiety, depression, and ASD\cite{173,174,175–176}. In rodents, activation of 5-HT receptors in different brain regions has been implicated in long-term emotional alterations. Thus, in the early-life stress model of maternal separation, an enhanced adult 5-HT2A/C-mediated prefrontal function was found\cite{62}, while the early postnatal pharmacological blockade of these receptors prevented the emergence of the anxiety phenotype in the same model\cite{63}. Interestingly, the expression of 5-HT2A/C receptors appeared to be substantially modified by prenatal stress but not after maternal separation\cite{55,63}. Emotional alterations produced by the postnatal exposure to SSRIs also appear to be mediated, at least in part, by 5-HT2A/C receptors. That is, the exposure to 5-HT2A and 5-HT2C antagonists during the early postnatal period prevents the anxiety and depressive-like phenotypes induced by fluoxetine, while the treatment in the same period with agonists of the same receptors produces anxiety\cite{104}.

Other 5-HT receptors such as the 5-HT1A and 5-HT7 were also implicated in the behavioral consequences of early postnatal SSRIs. That is, some of the adult emotional effects induced by postnatal fluoxetine were found to be enhanced by the co-treatment with a 5-HT1A receptor agonist\cite{116}. More recently, the prefrontal 5-HT7 receptors have been shown to have a crucial developmental role in the emergence of anxiety and depressive-like symptoms in the model of postnatal fluoxetine\cite{112}. Additionally, increased stimulation of 5-HT1A receptors during early postnatal life was also shown to mimic deficits produced by early SSRIs on adult social interactions of relevance to ASD\cite{128}. On the other hand, in the VPA model, activation of 5-HT1A receptors improves some of the social and cognitive deficits produced in that model\cite{166}.

Accumulating evidence indicates a primordial role of the prefrontal cortex (PFC) in these neurodevelopmental mechanisms. Thus, disruptions of developmental processes in the PFC such as circuit formation/refinement, synaptic connectivity, and oligodendrogenesis/myelination, have been directly linked to the early vulnerability to stress-related and emotional alterations (Fig. 1). Prenatal stress has been reported to produce a decrease in spine density in mPFC pyramidal neurons\cite{68}. A similar
reduction was described in layer 2–3 mPFC pyramidal cells after postnatal exposure to fluoxetine, accompanied by altered excitability of this neuronal population and exuberant dendritic branching\textsuperscript{106,115}. Functional imaging analysis in trumpet-tailed rats subjected to maternal separation stress has shown a global decrease in brain activity in PFC circuits likely engaged in prefrontal-limbic control\textsuperscript{177}. Besides, decreases in the excitability of PFC pyramidal neurons have been recently described after maternal separation\textsuperscript{178}. Consistent findings were described when analyzing the expression of activity-related immediate early genes in the PFC in the same model, accompanied by a precocious oligodendrocyte differentiation, and hypo-myelination\textsuperscript{67,179,180}. In agreement with this, postnatal exposure to the SSRI citalopram, but not the prenatal treatment, was shown to alter the oligodendrocyte morphology and the callosal connectivity\textsuperscript{126}.

Recent studies have shown a synaptic hyper-connectivity of corticolimbic circuits such as the PFC-to-dorsal raphe nucleus (DRN) one, after postnatal exposure to fluoxetine during the early postnatal period\textsuperscript{111}. Furthermore, these changes were found to be mediated by the developmental 5-HT signaling through the 5-HT7 receptors in the PFC\textsuperscript{112}. Importantly, reciprocal circuits connect the PFC to DRN 5-HT neurons, and these pathways have a crucial role in controlling stress-coping strategies and emotional responses throughout life\textsuperscript{111,181,182}.

Direct inhibition of the activity of DRN 5-HT neurons using chemogenetic tools has been shown to prevent the emergence of the emotional alterations produced by

---

**Fig. 1** Schematic overview of some of the main effects reported after either stress, SSRI or VPA exposures during gestation and/or early postnatal life, on different brain regions and neurodevelopmental processes. Different rodent models of exposure to stress (forced swim, helplessness, social defeat, restraint, maternal separation) and to chemical substances (SSRIs, VPA) are shown. Main brain regions affected in these models are indicated (Olfactory bulb OB, Prefrontal cortex PFC, Lateral habenula LHb, Amygdala AMY, Hippocampus HIP, Ventral tegmental area VTA, Dorsal raphe nucleus DRN, Cerebellum CBL), together with summarized effects on neurodevelopmental molecular, cellular, and circuit mechanisms. These include hyperexcitability of glutamate neurons, exuberant glutamate synaptogenesis, decreases in dendrite and spine remodeling, decreased neurogenesis, reduction of the balance of glutamate/GABA transmission, reduced gliogenesis and myelination, and multiple changes in epigenetic control of gene expression.
postnatal fluoxetine. Additionally, maternal separation reduces the firing activity of DRN 5-HT neurons. Interestingly, gestational exposure to VPA was reported to delay the migration and differentiation of developing DRN 5-HT neurons.

Altogether, this evidence indicates a complex role of the developing 5-HT system and its targeted neural circuits, in the detrimental emotional effects produced by early-life exposures.

**Other neural circuits implicated**

Other studies have implicated other brain structures such as the ventral tegmental area (VTA) and lateral habenula (LHb), in the emotional vulnerability during early-life. Interestingly, postnatal SSRI – fluoxetine has been recently shown after SSRI exposure. On the other hand, the early-life experience of maternal separation was shown to perturb the communication within the network engaging hippocampal and PFC circuits.

Other studies point out to the amygdala as a key neural substrate involved in the effects of maternal separation and in utero VPA exposure. In utero VPA exposure (Fig. 1) has been described in the amygdala after VPA exposure. These investigations showed a reduced functional connectivity between the basolateral amygdaloid nucleus (BLA) and the PFC after maternal separation, in agreement with previous imaging findings in depressive patients. On the other hand, enhancements of the neuronal excitability and glutamate transmission in the amygdala have been reported in the VPA model. Besides, similar findings were described in the same model in frontocortical circuits. Interestingly, treatment with a metabotropic glutamate receptor 5 (mGluR5) antagonist rescued some of the social behavioral deficits observed in the VPA model. Additionally, changes on GABAergic neurochemical markers and modifications on the neuronal/glial cytoarchitecture, have been also reported in the amygdala and cerebellum, after prenatal exposure to VPA.

**Epigenetic mechanisms**

Other lines of evidence indicate an important role for epigenetic control of gene expression in the neurodevelopmental mechanisms at play in these preclinical models (Fig. 1). For instance, a transcriptional disruption of genes involved in developmental and immune gene networks has been described in the amygdala after VPA exposure. In other studies, researchers found that acetylation regulation of hippocampal gene expression by the histone deacetylase (HDAC) 4 is crucially involved in the adult emotional alterations caused by postnatal SSRIs. Consistent with its relevance, a decreased expression of several members of the HDAC family was reported in the amygdala after prenatal exposures to SSRIs or VPA. In the VPA model, alterations in cortical thickness have been consistently reported in the amygdala and cerebellum, after prenatal exposure to VPA. In the amygdala, after prenatal exposure to VPA, the expression of several members of the HDAC family was reported. Furthermore, in one of these studies the authors suggested that this mechanism could be a major contributor to the susceptibility/resilience to early-life stress, and the subsequent efficacy to antidepressant treatments.
been directly linked to its actions on the activity of HDACs. Thus, exposure to the HDAC inhibitor trichostatin A, can phenocopy many of the social behavioral deficits observed in the VPA model. Consistently, the prenatal treatment with valpromide, a VPA analog lacking the HDAC’s inhibitory activity, failed to reproduce the social interaction deficits.

Methylation of promoter regions has been also implicated in the effects of SSRIs during pregnancy and lactation. These investigations showed long-lasting changes in methylation levels of multiple genes in the hippocampus and cortex, including the Bdnf gene. Interestingly, chromatin remodeling and increased methylation levels in the PFC were reported, after either gestational stress or VPA exposure. Lastly, the transgenerational epigenetic inheritance of VPA-induced imbalance in excitatory/inhibitory transmission in the frontal cortex has been recently demonstrated, illustrating the rather complex repertoire of neurodevelopmental mechanisms that could be engaged in the different preclinical models of exposure.

Translational Aspects and Concluding Remarks

Rodent models have been very useful to investigate the mechanisms implicated in the development vulnerability to psychiatric disorders. Thus, preclinical research surveyed in this review allows the identification of neural substrates and neurobiological mechanisms impacted by early-life environmental exposures. Importantly, many of these findings may directly relate to what occurs in the complex human psychopathology. However, other biological processes linked to gene regulation and its possible interaction to environmental conditions, is growingly emerging as crucial actors involved in neuropsychiatric disorders. For instance, investigations in rodents with reduced 5-HT synthesis have shown a differential impact on the DRN 5-HT system of maternal separation, while reduction of the 5-HT transporter, SERT, leads to an enhanced anhedonia under similar stress conditions. Furthermore, combination of stress exposure and postnatal SSRI treatment in dams alters affective susceptibility of the offspring in a SERT-dependent manner. Conversely, behavioral alterations produced by in utero VPA exposure were not affected by the presence of SERT. On the other hand, rats with reduced SERT expression, and a previous history of maternal separation, showed improvements in stress-coping responses. Interestingly, in utero exposure to citalopram mitigates the detrimental effects of maternal stress on the fetal forebrain development, and these changes are thought to be mediated by normalizing brain 5-HT levels.

Human studies have shown that gestational exposure to SSRIs is associated with adverse neonatal outcomes that can be moderated by the SERT promoter polymorphism of the infants. Moreover, methylation of the SERT promoter can influence the soothability of infants that had exposition to SSRIs during the gestation. Additionally, the hyperserotoninemia and several stereotypical behavioral outcomes present in ASD patients can be replicated in mice that express a hyperfunctional SERT variant.

A critical point to be considered when analyzing the risk of exposure to SSRIs in pregnant women is the psychopathological state of the future mother. Evidence indicates that SSRI treatment in depressed mothers can prevent the modifications in brain’s connectivity produced in newborns that are exposed to unmedicated depressive mothers. In addition, other studies have shown that adverse effects produced by prenatal maternal depression on infant’s problematic temperament can be amplified by a concurrent prenatal traumatic stress.

In the case of VPA, human evidence supports only a poor interaction between the effects of prenatal exposure to VPA and maternal mental health. Thus, a very recent nationwide population-based analysis has shown that in utero exposure to VPA is associated with an increased risk of neurodevelopmental mental and behavioral disorders in exposed children. Moreover, these effects were stronger when exposures occurred during the second and third trimesters of pregnancy. However, these findings were not affected by the mother’s mental health.

Future investigations need to consider neurodevelopmental regulatory mechanisms as well as other biological factors, such as the neuroinflammatory and hormonal contexts, that could influence the brain’s homeostatic capacity to mitigate early-life environmental perturbations. Altogether, these further considerations will improve understanding of how developmental maladaptive mechanisms could increase the risk of vulnerability to mental disorders.

Acknowledgements

The authors are grateful to Drs. Delfina M. Romero, Anne Teissier, and Patricia Gaspar for their insightful comments on the paper. Our research is supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), the International Brain Research Organization (IBRO), and the CAEN program of the International Society for Neurochemistry. T.S.A. and C.V.A. are supported by Doctoral Fellowships from CONICET and M.S-R. is an investigator from the same institution.

Conflict of interest

The author declares no competing interests.

Publisher’s note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 20 October 2020 Revised: 16 April 2021 Accepted: 21 April 2021 Published online: 11 May 2021
Adjimann et al. Translational Psychiatry (2021)11:280

50. Richardson, H. N., Zorrilla, E. P., Mandyam, C. D. & Rivier, C. L. Exposure to repetitive versus varied stress during prenatal development generates two distinct anxiogenic and neuroendocrine profiles in adulthood. Endocrinology 147, 2506–2517 (2006).

51. Pallatets, M. E., Scacchi Bemessconi, P. A., Feleder, C. & Cutrera, R. A. Effects of prenatal stress on motor performance and anxiety behavior in Swiss mice. Physiol. Behav. 92, 951–956 (2007).

52. Zueva, A. R. et al. Prenatal restraint stress generates two distinct behavioral and neurochemical profiles in male and female rats. PLoS ONE 3, e2170 (2008).

53. Lakou, C. et al. Anxiety-like behaviour and associated neurochemical and endocrinological alterations in male pups exposed to prenatal stress. Psychoneuroendocrinology 27, 1646–1652 (2002).

54. Van den Hove, D. L. A. et al. Prenatal stress and subsequent exposure to chronic mild stress in rats: interdependent effects on emotional behavior and the serotoninergic system. Eur. Neuropsychopharmacol. 24, 595–607 (2014).

55. Akatsu, S., Ishikawa, C., Takemura, K., Ohtani, A. & Shiga, T. Effects of prenatal stress on maternal handling on anxiety, spatial learning and serotoninergic system of male offspring mice. Neurosci. Res. 101, 15–23 (2015).

56. Solani, F. C. et al. Unpredictable chronic prenatal stress and manifestation of generalized anxiety and panic in rat’s offspring. Prog. Neuropsychopharmacol. Biol. Psychiatry 85, 89–97 (2018).

57. Ituma-Neja, A. M., Arragada-Solimano, M., Lutette-Anders, A. & Dagrono-Subiaire, A. Effects of prenatal stress on anxiety- and depressive-like behaviours are sex-specific in prepubertal rats. J. Neuroendocrinol. 30, e12609 (2018).

58. Poltyrev, T., Gorodetsky, E., Bejar, C., Schorner-Apelbaum, D. & Weinstock, M. Effect of chronic treatment with laddostigil (TV-3326) on anxiogenic and depressive-like behaviour and on activity of the hypothalamic-pituitary-adrenal axis in male and female prenatally stressed rats. Psychopharmacology 181, 118–125 (2005).

59. Barbe-Shoshani, Y., Shoham, S., Bejar, C. & Weinstock, M. Sex-specific effects of prenatal stress on memory and markers of neuronal activity in juvenile rats. Dev. Neurosci. 38, 206–219 (2016).

60. Callaghan, B. L., Sullivan, R. M., Howell, B. & Tottenham, N. The international society for developmental psychobiology Sackler symposium: early adversity and the maturation of emotion circuits—a cross-species analysis. Front. Hum. Neurosci. 13, 167 (2019).

61. Uheński, M. L. & Fuchs, P. N. Maternal separation stress leads to enhanced emotional responses to noxious stimuli in adult rats. Behav. Brain Res. 212, 208–212 (2010).

62. Roque, S., Mesquita, A. R., Palha, J. A., Sousa, N. & Correa-Nevés, M. The behavioral and immunological impact of maternal separation: a matter of timing. Front. Behav. Neurosci. 8, 192 (2014).

63. Benekareddy, M., Vadodaria, K. C., Nair, A. R. & Vaidya, V. A. Postnatal serotonin neurons at an earlier time-point in females than in males. Front. Neurosci. 30, 1188 (2016).

64. Peña, C. J. et al. Early life stress disrupts social behavior and prefrontal cortex parvalbumin interneurons at an earlier time-point in females than in males. Neurosci. Lett. 566, 131–136 (2014).

65. Gardner, K. L., Thrivikraman, K. V., Lightman, S. L., Potsky, P. M. & Lovas, C. Y. Early life experiences alter the structure and function of the pituitary-adrenal axis. Neurosci. Biobehav. Rev. 33, 1252–1253 (2009).

66. Thomas, A. W., Caporale, N., Wu, C. & Wilbrecht, L. Early maternal separation impacts cognitive flexibility at the age of first independence in mice. Dev. Cogn. Neurosci. 18, 59–66 (2016).

67. Naninck, E. F. G. et al. Chronic early life stress alters developmental and adult neurogenesis and impairs cognitive function in mice. Hippocampus 25, 309–328 (2015).

68. Tchenio, A., Lecca, S., Valentino, K. & Maroni, M. Limiting habitual hyperactivity ameliorates maternal separation-driven depressive-like symptoms. Nat. Commun. 8, 1135 (2017).

69. Johannessen, C. U., Bakker, C., Horte, S. & Dyck, R. H. Behavioural outcomes of perinatal selective serotonin reuptake inhibitor exposure in mice. Sci. Rep. 7, 11401 (2017).

70. Teissier, A. et al. Early-life stress impairs postnatal oligodendrogenesis and adult emotional behaviour through activity-dependent mechanisms. Mol. Psychiatry 26, 1399–1413 (2021).

71. Rana, S., Pugh, P. C., Jackson, N., Clinton, S. M. & Kerman, I. A. Inborn stress reactivity shapes adult behavioral consequences of early-life maternal separation stress. Neurosci. Lett. 584, 146–150 (2015).

72. Qin, X. et al. Moderate maternal separation mitigates the altered synaptic transmission and neuronal activation in amygdala by chronic stress in adult mice. Mol. Brain 12, 111 (2019).

73. Rice, C. J., Sandman, C. A., Lenjay, M. R. & Baram, T. Z. A novel mouse model for acute and long-lasting consequences of early life stress. Endocrinology 149, 4892–4900 (2008).

74. Yang, X.-D. et al. Stress during a critical postnatal period induces region-specific structural abnormalities and dysfunction of the prefrontal cortex via CRF1. Neuropsychopharmacology. 40, 1203–1215 (2015).

75. Gallo, M. et al. Limited bedding and nesting induces maternal behavior resembling both hypervigilance and abuse. Front. Behav. Neurosci. 13, 157 (2019).

76. McAllister, B. B., Kiryanova, V. & Dyck, R. H. Behavioural outcomes of perinatal maternal stress exposure in mice. Neurosci. Lett. 566, 131–136 (2014).

77. Kroese, Y. et al. Perinatal reduction of functional serotonin transporters results in dysregulated stress-induced immediate early gene responses, and specific transcriptional changes that arise following early-life stress. Biol. Psychiatry 70, 1024–1032 (2011).

78. Velasquez, J. C. et al. In Utero exposure to citalopram mitigates maternal separation effects on fetal brain development. ACS Chem. Neurosci. 10, 3307–3317 (2019).

79. Harwood, A. J. Neurodevelopment and mood stabilizers. Curr. Mol. Med. 3, 225–240 (2003).

80. Ramsteijn, A. S. et al. Perinatal selective serotonin reuptake inhibitor exposure and behavioral outcomes: A systematic review and meta-analyses of animal studies. Neurosci. Biobehav. Rev. 114, 53–69 (2020).

81. Johannessen, C. U. Mechanisms of action of valproate: a commentatory. Neurochem. Int. 37, 103–110 (2000).

82. Velasquez, J. C. et al. In Utero exposure to citalopram mitigates maternal separation effects on fetal brain development. ACS Chem. Neurosci. 10, 3307–3317 (2019).

83. Hippocampus 25, 131–136 (2014).

84. Ramsteijn, A. S. et al. Perinatal selective serotonin reuptake inhibitor exposure and behavioral outcomes: A systematic review and meta-analyses of animal studies. Neurosci. Biobehav. Rev. 114, 53–69 (2020).

85. Kroese, Y. et al. Perinatal reduction of functional serotonin transporters results in developmental delay. Neuropharmacology 109, 96–111 (2016).

86. Nourlander, C. W. et al. Modulation of serotonin transporter function during fetal development causes disrupted heart cardiomyopathy and lifelong behavioral abnormalities. PLoS ONE 3, e2782 (2008).

87. Smit-Rijter, L. A. et al. Prenatal fluoxetine exposure induces life-long seroton 5-HT, receptor-dependent cortical abnormalities and anxiety-like behaviour. Neuropharmacology 62, 865–870 (2012).

88. McAllister, B. B., Kryanova, V. & Dyck, R. H. Behavioural outcomes of perinatal maternal fluoxetine treatment. Neurosci. Lett. 226, 356–366 (2012).
Ko, M.-C., Lee, L. J.-H., Li, Y. & Lee, L.-J. Long-term consequences of neonatal treatment of rat neonates significantly reduces oxidative stress in the hippocampus and in behavioral indicators of anxiety in adult offspring. *Front. Behav. Neurosci.* 67, 526–527 (2020).

Ishiwata, H., Shiga, T. & Okado, N. Selective serotonin reuptake inhibitor treatment of early postnatal mice reverses their prenatal stress-induced brain function. *Neuroscience* 133, 893–901 (2005).

Papa, D., Léna, C., Alexandre, C. & Adrien, J. Lasting syndrome of depression produced by reduction in serotonin uptake during postnatal development: evidence from sleep, stress, and behavior. *J. Neurosci.* 28, 3546–3554 (2008).

Ansorge, M. S., Morelli, E. & Gingrich, J. A. Inhibition of serotonin but not norepinephrine transport during development produces delayed, persistent perturbations of emotional behaviors in mice. *J. Neurosci.* 28, 199–207 (2008).

Maciag, D., Williams, L., Coppping, D. & Paul, I. A. Neonatal clonazepam exposure produces lasting changes in behavior which are reversed by adult imipramine treatment. *Eur. J. Pharmacol.* 532, 265–269 (2006).

Maciag, D. et al. Neonatal antidepressant exposure has lasting effects on behavior and serotonin circuitry. *Neuropsychopharmacology* 31, 47–57 (2006).

Harris, S. S., Maciag, D., Simpson, K. L., Lin, R. C. S. & Paul, I. A. Dose-dependent effects of neonatal SSRI exposure on adult behavior in the rat. *Brain Res.* 1429, 52–60 (2012).

Altiere, S. C. et al. Perinatal vs. genetic programming of serotonin states associated with anxiety. *Neuropsychopharmacology* 40, 1456–1470 (2015).

Simpson, K. L. et al. Perinatal antidepressant exposure alters cortical network function in rodents. *Proc. Natl Acad. Sci. USA* 108, 18465–18470 (2011).

Rodríguez-Porcel, F. et al. Neonatal exposure of rats to antidepressants affects behavioral reactions to novelty and social interactions in a manner analogous to autistic spectrum disorders. *Anat. Rec. Hoboken* 294, 1726–1735 (2011).

Khatri, N., Simpson, K. L., Lin, R. C. S. & Paul, I. A. Lasting neurobehavioral abnormalities in rats after neonatal activation of serotonin 1A and 1B receptors: possible mechanisms for serotonin dysfunction in autistic spectrum disorders. *Psychopharmacology* 231, 1191–1200 (2014).

Freund, N., Thompson, B. S., Demorandie, J., Vaccaro, K. & Andersen, S. L. Windows of vulnerability: maternal separation, age, and fluoxetine on adolescent depressive-like behavior in rats. *Neuroscience* 249, 88–97 (2013).

Rayen, I., van den Hove, D. L., Prickaerts, J., Steinbusch, H. W. & Pavluski, J. L. Fluoxetine during development reverses the effects of prenatal stress on depressive-like behavior and hippocampal neurogenesis in adolescence. *PloS ONE* 6, e24003 (2011).

Safar, A. A., Fatehi-Charahlar, L., Motayeghien, N. & Homberg, J. R. Fluoxetine normalizes the effects of prenatal maternal stress on depression- and anxiety-like behaviors in mouse dams and male offspring. *Behav. Brain Res.* 311, 354–367 (2016).

Zohar, I., Shoham, S. & Weinstock, M. Perinatal citalopram does not prevent the effect of prenatal stress on anxiety, depressive-like behavior and serotonin transmission in adult rat offspring. *Eur. J. Neurosci.* 43, 590–600 (2016).

Wagner, G. C., Reuhl, K. R., Cheh, M., McRae, P. & Halliday, A. A. K. A new neurobehavioral model of autism in mice: pre- and postnatal exposure to valproic acid animal model of autism. *Neuropsychopharmacology* 30, 80–89 (2005).

Hou, Q. et al. A developmental study of abnormal behaviors and altered GABAergic signaling in the VPA-treated rat model of autism. *Front. Behav. Neurosci.* 12, 182 (2018).

Narita, M. et al. Nonequiequipefne movement and behavioral alterations in a thalidomide or valproic acid-induced autism model rat. *Neurosci. Res.* 66, 2–6 (2010).

Kataoka, S. et al. Autism-like behaviors with transient histone hyper-acetylation in mice treated prenatally with valproic acid. *Int J. Neuropsychopharmacol.* 16, 91–103 (2013).

Mehta, M. V., Gandhi, M. J. & Siegel, S. J. mGlu5-antagonist mediated reversal of elevated stereotyped, repetitive behaviors in the VPA model of autism. *PloS ONE* 6, e20277 (2011).
137. Schneider, T., Turkzw, J. & Przewlocki, R. Environmental enrichment reverses behavioral alterations in rats prenatally exposed to valproic acid: issues for a therapeutic approach in autism. *Neuropsychopharmacology* **31**, 36–46 (2006).

138. Schneider, T. et al. Gender-specific behavioral and immunological alterations in an animal model of autism induced by prenatal exposure to valproic acid. *Psychoneuroendocrinology* **33**, 728–740 (2008).

139. Tsai, N. et al. Abnormality of cardiac rhythm accompanied by an increase in frontal cortex serotonin in animal model of autism. *Neurosci. Res.* **57**, 289–295 (2007).

140. Markram, K., Rinaldi, T., La Mendola, D., Sandi, C. & Markram, H. Abnormal fear conditioning and amygdala processing in an animal model of autism. *Neuropsychopharmacology* **33**, 901–912 (2008).

141. Bambini-Junior, V. et al. Animal model of autism induced by prenatal exposure to valproate: behavioral changes and liver parameters. *Brain Res.* **1408**, 8–16 (2011).

142. Mychasiuk, R., Richards, S., Nakahashi, A., Kolb, B. & Gribb, R. Effects of rat prenatal exposure to valproic acid on behaviour and neuro-anatomy. *Dev. Neurosci.* **34**, 266–276 (2012).

143. Bambini-Junior, V. et al. Animal model of autism induced by prenatal valproic acid treatment. *Neuroscience* **434**, 8–21 (2020).

144. Kim, K. C. et al. The critical period of valproate exposure to induce autistic symptoms in Sprague-Dawley rats. *Behav. Brain Res.* **201**, 137–142 (2011).

145. Schneider, T. K. C. et al. The critical period of valproate exposure to induce autistic symptoms in Sprague-Dawley rats. *Toxicol. Lett.* **210**, 137–142 (2011).

146. Schulze, H., C., Et al. Increased anxiety-like behavior and altered GABAergic system in the amygdala and cerebellum of VPA rats—an animal model of autism. *Neurosci. Lett.* **629**, 9–14 (2016).

147. Barrett, C. E. et al. Developmental disruption of amygdala transcriptome and sociocommunicative behavior in rats exposed to valproic acid prenatally. *Mol. Autism* **8**, 28 (2017).

148. Rouillet, F. I., Wollsot, L., DeGanzo, D. & Foster, J. A. Behavioral and molecular characterization of a new model of autism induced by prenatal exposure to the anti-epileptic drug valproic acid. *Neuroscience* **170**, 514–522 (2010).

149. Mayer, L. C. et al. Male-specific alteration in excitatory post-synaptic development and social interaction in pre-natal valproic acid exposure model of autism spectrum disorder. *J. Neurochem.* **124**, 852–843 (2013).

150. Faivre, M. R. et al. Predictable enriched environment prevents development of hyper-emotionality in the VPA rat model of autism. *Front. Neurosci.* **9**, 127 (2015).

151. Raza, S. et al. Effects of prenatal exposure to valproic acid on the development of juvenile-typical social play in rats. *Behav. Pharmacol.* **26**, 707–719 (2015).

152. Kaczorowska, N., Serfe, A., Campolongo, M., Zappella, C. & Depino, A. Male-specific effects of prenatal valproic acid exposure on sociability and neuro-inflammation: Relevance for susceptibility and resilience in autism. *Psychoneuroendocrinology* **110**, 104441 (2019).

153. Gielko, K. et al. Valproic acid exposure impairs ultrasonic communication in infant, adolescent and adult rats. *Eur. Neuropsychopharmacol.* **41**, 52–62 (2020).

154. Liu, M. et al. Abnormal reinforcement learning in a mouse model of autism induced by prenatal exposure to valproic acid. *Behav. Brain Res.* **395**, 112836 (2020).

155. Sui, L. & Chen, M. Prenatal exposure to valproic acid enhances synaptic plasticity in the medial prefrontal cortex and fear memories. *Brain Res. Bull.* **87**, 556–563 (2012).

156. Wang, C.-C. et al. S-HT1A-receptor agonist modified amygdala activity and amygdala-associated social behavior in a valproate-induced rat autism model. *Int. J. Neuropsychopharmacol.* **16**, 2007–2039 (2013).

157. Acosta, J. et al. Deficits in temporal processing in mice prenatally exposed to Valproic Acid. *Eur. J. Neurosci.* **47**, 619–630 (2018).

158. Chomiak, I., Nak, V., Block, E. & Hui, B. Altering the trajectory of early postnatal cortical development can lead to structural and behavioural features of autism. *BMC Neurosci.* **11**, 102 (2010).

159. Norton, S. A. et al. Long-lasting behavioral and neuroanatomical effects of prenatal valproic acid treatment. *Neuroscience* **434**, 8–21 (2020).

160. Yochum, C. L., Dowling, P., Reuhl, K. R., Wagner, G. C. & Ming, X. VPA-induced apoptotic and behavioral deficits in neonatal mice. *Brain Res.* **1203**, 126–132 (2008).

161. Mony, T., J., Lee, J. W., Dreyfus, C., DiCicco-Bloom, E. & Lee, H. J. Valproic Acid Exposure during Early Postnatal Gliogenesis Leads to Autistic-Like Behaviors in Rats. *Clin. Psychopharmacol. Neurosci.* **14**, 338–344 (2016).

162. Hanley, H. G., Stah, S. M. & Freedman, D. H. Hypersertorinemia and amine metabolites in autistic and retarded children. *Arch. Gen. Psychiatry* **34**, 521–531 (1977).

163. Lesch, K. P. et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* **274**, 1527–1531 (1996).

164. Hariri, A. R. et al. Serotonin transporter genetic variation and the response of the serotonin transporter gene regulatory region. *Science* **274**, 400–403 (2002).

165. Caspi, A. et al. Influence of life stress on depression: moderation by a polymorphism in the S-HTT gene. *Science* **301**, 386–389 (2003).

166. Coutinho, A. M. et al. Variants of the serotonin transporter gene (SLC6A4) significantly contribute to hypersertorinemia in autism. *Mol. Psychiatry* **9**, 264–271 (2004).

167. Bock, J., Reedl, A. & Braun, K. Differential changes of metabolic brain activity and interregional functional coupling in prefronto-limbic pathways during different stress conditions: functional imaging in freely behaving rodent pups. *Front. Cell. Neurosci.* **6**, 19 (2012).

168. Sun, X., Zhang, Y., Li, X., Liu, X. & Qin, C. Early-life neglect alters emotional and cognitive behavior in a sex-dependent manner and reduces glutamatergic neuronal excitability in the prefrontal cortex. *Front. Psychol.* **11**, 572224 (2020).

169. Sood, A., Pali, S., Bhattacharya, A., Chaudhuri, K. & Vaidya, V. A. Early emergence of altered S-HT2A receptor-evoked behavior, neural activation and gene expression following maternal separation. *Int. J. Dev. Neurosci.* **65**, 21–28 (2018).

170. Yang, Y. et al. Neonatal maternal separation impairs prefrontal cortical myelination and cognitive functions in rats through activation of Wnt signaling. *Cereb. Cortex* **27**, 2887–2894 (2017).

171. Amat, J. et al. Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. *Nat. Neurosci.* **8**, 565–571 (2005).

172. Warden, M. R. et al. A prefrontal cortex-brainstem neuronal projection that controls response to behavioural challenge. *Nature* **492**, 438–432 (2012).

173. Villela, F. C. et al. Maternal separation increases pain sensitivity by reducing the activity of serotoninergic neurons in the dorsal raphe nucleus and noradrenergic neurons in locus coeruleus. *Neurosci. Lett.* **748**, 153574 (2021).

174. Miyaizaki, K., Nitta, N. & Nitta, M. Maternal administration of thalidomide or valproic acid causes abnormal serotoninergic neurons in the offspring: implications for pathogenesis of autism. *Int. J. Dev. Neurosci.* **23**, 287–297 (2005).

175. Sperry, J. et al. Early life stress-induced alterations in the activity and morphology of ventral tegmental area neurons in female rats. *Neuropsychopharmacology* **13**, 100250 (2020).

176. Muszala, A., Scorto-Lomassese, S., Bernard, J. F., Soiza-Reilly, M. & Gaspar, P. Conditional anterograde tracing reveals distinct targeting of individual serotonin cell groups (B5–B9) to the forebrain and brainstem. *Brain Struct. Funct.* **221**, 535–561 (2016).
