Long-Term Outcomes of Developmental Exposure to Fluoxetine: A Review of the Animal Literature

Veronika Kiryanova^{a, c} Brendan B. McAllister^{a, c} Richard H. Dyck^{a–c}Departments of ^aPsychology and ^bCell Biology and Anatomy and ^cHotchkiss Brain Institute, University of Calgary, Calgary, Alta., Canada

Key Words

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Abstract

During and following pregnancy, women are at high risk of experiencing depression, for which fluoxetine (FLX; brand names Prozac, Sarafem, Rapiflux) is the most commonly prescribed treatment. An estimated 1.4–2.1% of pregnant women use this medication, which inhibits the reuptake of serotonin and thereby increases serotonergic activity at the synapse. Serotonin acts as a cue guiding numerous neurodevelopmental processes, and changes in the concentration of serotonin can disrupt normal in utero brain development and organization in humans and other animals, thus providing a mechanism by which maternal intake of FLX might alter neural development and ultimately behaviour. Despite this possibility, long-term alterations of behaviour and the brain have not been well studied in individuals exposed to FLX during pregnancy or soon after birth, perhaps because conducting such studies beyond infancy presents significant challenges. To remedy this problem, many researchers have turned to modelling the effects of developmental FLX exposure in non-human animals, primarily rodents. The body of literature on this topic has expanded considerably over the past several years, yet a comprehensive review is lacking. In

order to fill this gap, we have summarized the findings of those studies describing the long-term behavioural and neurophysiological effects of FLX exposure in non-human animals in early development. We also discuss methodological considerations and common shortcomings of research in this area. The precise nature of the long-term effects of developmental FLX exposure remains difficult to specify, as these effects appear to be highly variable and dependent on numerous factors. Overall, however, it is clear that early FLX exposure in non-human animals can alter the development of the brain in ways that are relevant to behaviour in adulthood, decreasing exploration and social interaction, and in some cases altering anxiety- and depression-like behaviours.

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Introduction

A significant number of infants are exposed to selective serotonin reuptake inhibitor (SSRI) medications prior to birth or while nursing. Women of child-bearing age are the population at highest risk of experiencing depres-

Veronika Kiryanova and Brendan B. McAllister contributed equally to this work.

sion [1], for which SSRIs are the treatment of choice. Overall, over 5% of pregnant women use SSRIs during pregnancy [2–4]. Fluoxetine (FLX; also known by the brand names Prozac, Sarafem, and Rapiflux), one of the most widely used SSRIs, is taken by 1.4–2.1% of all pregnant women throughout or at some point during pregnancy [2–4]. FLX and its metabolite norfluoxetine (NFLX) cross the placental barrier [5] and are excreted in breast milk, resulting in plasma concentrations that can reach therapeutic levels in breastfed infants [6]. To date, a considerable amount of research has been conducted on the effects of exposure to SSRIs during development. The majority of this work has focused on exposure to SSRIs in general, with significantly fewer studies focusing specifically on the effects of FLX or other individual SSRIs. In all cases, however, there has been a complete lack of focus on the outcomes of perinatally exposed individuals beyond the preschool age [7]. This is concerning, given that serotonin modulates a multitude of developmental processes [for a review, see 8], and that genetic variation in the availability of serotonin is known to alter the organization of the brain as well as behaviour later in life [9]. To address this concern, many laboratories have turned to modelling the developmental effects of SSRI exposure in non-humans. As in humans, FLX crosses the placental barrier in rats and mice, producing a comparable level of fetal exposure [10, 11]. It also crosses the rodent fetal blood-brain barrier, leading to detectable FLX and NFLX levels in the blood and brain of pups (online suppl. table 1; for all online suppl. material, see www.karger.com/doi:10.1159/000355709). Therefore, these animals can serve as useful models of SSRI exposure, allowing for the investigation of parameters that would not easily be probed in humans, particularly neurodevelopmental and long-term behavioural outcomes. As this review will make apparent, however, there is still a great deal of discrepancy amongst the results of studies in this area. This likely results from many factors, including: differences in the dosage and route of administration; the length of exposure and the period in development over which it occurs; the species, strain, and sex of the animal used; the age of the animal during exposure and at testing; the animal's history of exposure to stress and handling; the behavioural testing protocol; and, of course, differences in the SSRI itself. To narrow the scope of this review, we have elected to include only those studies examining the effects of exposure to FLX. This review, therefore, represents only a portion (though a large one) of the broader literature on the effects of developmental SSRI exposure in non-human animals.

Teratogenic and Early Developmental Effects

Teratogenic and early postnatal outcomes have rarely been a focus in most studies of developmental FLX exposure in non-human animals. This is perhaps because these are the effects most easily studied in human infants. Nevertheless, several studies have provided such data, and the pattern is remarkably similar to what has been observed by clinical researchers. The majority of the clinical studies have not detected an increase in the risk of developing major malformations, following fetal exposure to all SSRIs considered together or to FLX specifically [for a review of the subject, see 12]. However, there are a few notable exceptions. One study found that women who were prescribed FLX during pregnancy gave birth to infants who were at higher risk of developing cardiovascular abnormalities, specifically ventricular septal defects, but were not at a higher overall risk for other malformations [13]. Another study found an association between first-trimester FLX exposure and cardiovascular anomalies, but again not with other malformations [14]. On the other hand, a third study found a contrasting pattern, where there was a small increase in the overall risk for major malformations in children of women who reported FLX use during pregnancy, but no increase in the risk of cardiovascular defects specifically [15], and another study found an increase in the risk of developing septal heart defects following exposure to other SSRIs, but not FLX in particular [16]. Additionally, it appears that FLX-exposed infants may be more likely to display persistent pulmonary hypertension after birth, though the absolute risk level remains low even following SSRI exposure [17]. Unfortunately, none of these studies were able to control for the potential effects of maternal depression, which makes it difficult to be certain that FLX is the true cause of these malformations. In studies of non-human animals, few major malformations have been reported, though many researchers, especially those interested in long-term effects, likely do not examine for these all that closely. However, as with studies of human infants, a few have noted cardiovascular abnormalities. In one study, exposure to FLX from mid-to-late gestation in rats caused pulmonary hypertension and structural abnormalities of the heart and pulmonary vascular system, coinciding with an increase in postnatal mortality from 0 in controls to 15% in exposed pups [18]. A similar increase was noted by Vorhees et al. [19] following prenatal exposure, and by Müller et al. [20] following administration of a high dose of FLX to the dam from 1 week after conception to weaning, though lower doses

did not have the same effect. In neither study was the cause of this increased mortality examined. However, a related finding in mice, wherein FLX exposure beginning after the first week of gestation resulted in a very dramatic rate of mortality of 81%, was linked to an abnormality of the heart, specifically dilated cardiomyopathy [10]. Oddly, the high mortality rate noted in this study, despite resulting from a very low maternal dosage (0.8 mg/kg/day, i.p.), has not been consistently replicated in numerous other studies using much larger doses over a similar period of time during development [21–23]. Together, these findings suggest that such a small dose only has a pronounced effect when administered by intraperitoneal injection, and more broadly, that increased mortality might result only from exposure during a critical period in development, perhaps organogenesis, though it should be noted that some researchers who reported no change in mortality began administration as early as the sixth gestational day [21, 23]. In addition to cardiac malformations, minor abnormalities and developmental delays have also been noted in FLX-exposed rats, such as transient impairment of the negative geotaxis reflex and delayed eruption of the incisor teeth [23], as well as an increase in the rate of ultrasonic vocalizations [24]. Unfortunately, these findings have not been well corroborated, as the same measures have not commonly been examined across studies. Delays in reflex development may have some relation to a subtle impairment in fine motor skill noted in children between the ages of 6 and 40 months who were exposed to FLX during pregnancy [25], which is one of the very few effects of developmental exposure noted beyond early infancy. Whether or not this impairment was persistent or transient is not known, as no follow-up testing was conducted. Finally, sexual differentiation during early life may also be altered, as postnatally exposed male (but not female) rats were found to have shorter than normal anogenital distances at 3 weeks of age, though serum testosterone was not altered, and the difference in anogenital distance was no longer apparent by adulthood in males [26]. In another study, prenatal exposure to low and high doses of FLX was found to have no effect on anogenital distance at birth [20].

As in the case of congenital malformations, it is still an open question whether prenatal FLX exposure affects factors such as gestational length, birth weight, and litter size. Some clinical studies have reported decreases in gestational length and birth weight following maternal FLX exposure [27, 28], though the majority have not [as reviewed in 12, 29]. However, it is worth noting that such

findings are often difficult to interpret, as the effect of FLX exposure can be confounded by the effect of maternal depression, which itself might increase the risk of premature birth [30]. Maternal condition is more easily controlled for in studies of non-human animals, some of which have found that FLX has a deleterious effect on one or more measures, including decreased pregnancy duration [22], litter size [11, 31] and birth weight [19, 20, 22–24, 32]. However, as with the clinical research, for each of these factors there are multiple studies that report no significant alterations, even using similar dosages and periods of exposure (table 1). Thus, straightforward conclusions are difficult to draw, a problem that is exacerbated by the fact that not all studies include these relatively simple measures.

Another interesting, although limited, line of research is the effect of prenatal FLX exposure in sheep. FLX was administered by a method of intravenous infusion into the ewe, producing serum levels within the human therapeutic range, and the fetuses were assessed. FLX exposure decreased the time spent in the low-voltage electrocortical/rapid eye movement behavioural state [33] and increased the levels of stress hormones, indicating disruption of hypothalamic-pituitary-adrenal axis function [34]. Gestational age and birth weight were not altered.

Long-Term Behavioural Outcomes

Due to the role of serotonin in development, it is not surprising that FLX exposure can exert developmental effects. Whether or not these effects are behaviourally significant and persist into maturity is, however, another question. In human infants, little research has been done to address this question, as FLX-exposed infants have not been examined beyond the age of 7 years [as reviewed in 7]. Fortunately, one of the strengths of modelling FLX exposure in non-human animals is that the effects of exposure during development on the behaviour of mature animals can be relatively easily assessed. Numerous studies have been conducted on just this topic.

Depression and Anxiety

As an indicator of depression-like behaviour, several studies have assessed the amount of time spent immobile in the forced swim test. In this test, the animal is placed in a stressful, inescapable situation, and immobility is considered to be a form of ‘behavioural despair’ where the animal ceases its attempts to escape. There appear to be

Table 1. Teratogenic effects of developmental exposure to FLX

	Species	Exposure period	Dosage, mg/kg/day	Administration route	Reference
<i>Gestational length</i>					
↓	rat	E15–E20	8, 16	IG	22
↔	rat	E13–E20	5, 10	SQ	24
	rat	E6–E20	8, 12	IG	23
	rat	E7–E20	1, 5, 12	IG	19
	rat	E7–P21	0.4, 1.7, 17	IG to dam	20
	mouse	pregnancy	10	DW	31
<i>Mortality</i>					
↑	rat	E11–E21	10	IG	18
	rat	E7–E20	12	IG	19
	rat	E7–P21	0.4, 1.7, 17	IG to dam	20
	mouse	E8–E18	0.3, 0.6, 0.8	IP	10
↔	rat	E7–E20	1, 5	IG	19
	rat	E15–E20	8, 16	IG	22
	rat	E6–E15	2, 5, 12.5	IG	21
	rat	E6–E20	8, 12	IG	23
<i>Litter size</i>					
↓	rat	E11–E21	12	IG	11
	mouse	pregnancy	10	DW	31
↔	rat	E13–E20	10	SQ	32
	rat	E13–E20	5, 10	SQ	24
	rat	E15–E20	8, 16	IG	22
	rat	E6–E20	12	IG	23
	rat	E7–E20	1, 5, 12	IG	19
	rat	E7–P21	0.4, 1.7, 17	IG to dam	20
	rat	E14–P7	10	OMP to dam	39
	mouse	E0–P21	7.5	IG to dam	35, 66
<i>Birth weight</i>					
↓	rat	E7–E20	12	IG	19
	rat	E6–E20	12	IG	23
	rat	E13–E20	10	SQ	32
	rat	E13–E20	5, 10	SQ	24
	rat	E15–E20	8, 16	IG	22
	rat	E7–P21	0.4, 1.7, 17	IG to dam	20
↔	rat	E6–E20	8	IG	23
	rat	E11–E21	10	IG	18
	rat	E6–E15	2, 5, 12.5	IG	21
	rat	E7–E20	1, 5	IG	19
	rat	E14–P7	10	OMP to dam	39
	rat	E0–P21	5	IG to dam	47
	mouse	E8–E18	0.3, 0.6, 0.8	IP	10
	mouse	E0–P21	7.5	IG to dam	35, 66

Arrows indicate direction of change; ↔ = no change. IP = Intraperitoneal; SQ = subcutaneous; DW = drinking water; OMP = osmotic mini-pump; IG = intragastric gavage; E = embryonic day; P = postnatal day.

sex differences in the effect of early FLX exposure on this behaviour, as female, but not male, mice exposed to FLX throughout pregnancy and until weaning show an increase in immobility when tested during adolescence and adulthood [35]. However, the conclusion that FLX exposure increases depression is obscured by the finding that exposed female mice take longer to become immobile [36], which is associated with a decrease in depression, though the larger dosage, more restricted period of exposure, and non-stressful method of administration used in this study might account for the difference in findings. In addition, it has been observed that male FLX-exposed mice spend less time immobile [37], though it is difficult to compare this finding to the previous two, as FLX exposure was exclusively postnatal and administered directly to the pups via a stressful method (injection) rather than indirectly by maternal administration. Other studies examining exclusively prenatal or exclusively postnatal exposure have failed to detect changes in immobility [11, 38] (tables 2, 3).

Changes in anxiety-like behaviour have also been examined, most commonly using the open-field test (OFT) and elevated plus maze (EPM), but also using the novelty-suppressed feeding/drinking tests. The bulk of the evidence indicates that FLX exposure either does not alter anxiety in adolescent or adult animals [23, 35, 37, 39–43] or causes an increase [10, 11, 44–46]. Interestingly, it appears that the novelty-suppressed feeding/drinking tests are particularly sensitive to the effects of FLX exposure, as differences have been found in these tests without observing changes in the EPM or OFT [11, 44, 45]. Of the studies that did observe increased anxiety in the EPM or OFT, one used a sample that could be considered exceptional due to the uncommonly high mortality rate, discussed above [10], and the other used a non-standard stress-preceded version of the EPM in addition to the standard EPM. In the standard EPM, no differences were observed [11]. To our knowledge, only 2 studies in this area have noted a decrease in anxiety. In one, female offspring exposed to FLX by non-stressful maternal administration showed decreased anxiety in the EPM, though not in the OFT [36]. In the other, adolescent, but not adult, male and female rats exposed to FLX from gestation through to weaning showed decreased anxiety in the novelty-suppressed feeding test [47]. There do not appear to be any obviously identifiable experimental factors that can account for these disparate findings. However, it is possible that differences may have resulted from seemingly minor variations in behavioural testing methodology. For example, in the EPM prior handling is known to

Table 2. Effects of developmental exposure to FLX on the behaviour of adolescent animals (up to postnatal day 49)

	Species and sex (if not both)	Exposure period	Dosage, mg/kg/day	Administration route	Reference
<i>Weight</i>					
↑	rat	E11–E21	12	IG	11
↓	mouse	P4–P21	10	IP to pup	37
↔	rat	E13–E20	10	SQ	32
	mouse	E8–E18	0.3, 0.6, 0.8	IP	10
<i>Anxiety</i>					
<i>Elevated plus maze</i>					
↑	mouse	E8–E18	0.3, 0.6, 0.8	IP	10
↔	rat	E6–E20	8, 12	IG	23
	rat (M)	P0–P4	20	SQ to pup	41
	mouse	E0–P21	7.5	IG to dam	35
<i>Novelty-suppressed feeding</i>					
↓	rat	E0–P21	5	IG to dam	47
<i>Exploration</i>					
↓	rat	E6–E20	8, 12	IG	23
	rat	P0–P6	10	SQ to pup	50
	rat (M)	P0–P4	20	SQ to pup	41
	rat (M)	E0–P21	7.5	IG to dam	51
	mouse (M)	E0–P21	7.5	IG to dam	35
↔	rat	E6–E20	8, 12	IG	23
	rat	E7–E20	1, 5	IG	19
	rat	E13–E20	5, 10	SQ	24
	rat (M)	P0–P4	20	SQ to pup	41
	rat	P1–P21	5	OMP	38
	mouse	E8–E18	0.3, 0.6, 0.8	IP	10
	mouse (F)	E0–P21	7.5	IG to dam	35
<i>Depressive behaviour</i>					
↑	mouse (F)	E0–P21	7.5	IG to dam	35
↔	rat	P1–P21	5	OMP	38
	mouse (M)	E0–P21	7.5	IG to dam	35
<i>Acoustic startle</i>					
↔	rat	E7–E20	1, 5, 12	IG	19
<i>Motor skill</i>					
↑	rat	E6–E20	8, 12	IG	23
↓	rat (M)	P0–P4	20	SQ to pup	41
<i>Thermal threshold</i>					
↑	rat	P0–P6	10	SQ to pup	50
	guinea pig	pregnancy	7	OMP	54
↔	rat	E21–P21	10	oral to dam	53
	mouse	E0–P21	7.5	IG to dam	35
<i>Sensorimotor function</i>					
↓	rat	P0–P6	10	SQ to pup	50
<i>Social behaviour (play)</i>					
↓	rat	E11–E21	12	IG	11
	rat (M)	P8–P21	10	SQ to pup	52
↔	rat (F)	P8–P21	10	SQ to pup	52
<i>Spatial memory</i>					
↑	rat	E6–E20	8, 12	IG	23
↔	rat	E7–E20	1, 5, 12	IG	19

	Species and sex (if not both)	Exposure period	Dosage, mg/kg/day	Administration route	Reference
<i>Fear memory</i>					
↓	rat	E6–E20	12	IG	23
↔	rat	E6–E20	8	IG	23
	rat	E7–E20	1, 5, 12	IG	19
<i>Novel object exploration</i>					
↓	rat (M)	P8–P21	10	SQ to pup	52
↔	rat (F)	P8–P21	10	SQ to pup	52

Arrows indicate direction of change; ↔ = no change. IP = Intraperitoneal; SQ = subcutaneous; DW = drinking water; OMP = osmotic mini-pump; IG = intragastric gavage; E = embryonic day; P = postnatal day.

exert considerable effects [48, 49], which means that the temporal position of the EPM within the test battery and the animals' history of handling or stress could shape the results. This point is further emphasized by the fact, mentioned above, that differing results have been obtained depending on the level of stress of the animal being tested in the EPM.

Locomotion, Motor Coordination, Sensorimotor Function, and Circadian Rhythmicity

The effect of developmental FLX exposure on exploratory locomotion has been widely examined, with results that show greater consistency than any other behavioural domain so far assessed. In adolescence, early exposure inhibits locomotion [23, 41, 50, 51] and decreases novel object exploration [52]. Lisboa et al. [35] observed decreased locomotion only in male mice, suggesting a difference in sensitivity between the sexes. Moreover, this hypolocomotion appears to persist into adulthood, and does not depend on whether FLX exposure is prenatal or postnatal, or whether FLX is administered directly to the pup or indirectly via the dam [23, 37, 39, 40, 44, 45]. Though some other studies have failed to detect an effect (tables 2, 3), to our knowledge no study has reported an increase in locomotion following developmental FLX exposure.

Interestingly, FLX exposure may affect not just the quantity of motor activity, but its quality as well, though the evidence for this is mixed. Bairy et al. [23] noted that male and female rats exposed prenatally to FLX performed better on a test of motor coordination in which the rat had to walk on a rotating cylinder. However, Lee and Lee [41] found that male adolescent rats briefly ex-

Table 3. Effects of developmental exposure to FLX on the behaviour of adult animals (from postnatal day 56 on)

	Species and sex (if not both)	Exposure period	Dosage, mg/kg/day	Administration route	Reference
<i>Weight</i>					
↓	rat	E13–E20	10	SQ	24
	mouse	P4–P21	10	IP to pup	37
	mouse (F)	E15–P12	25	DW to dam	36
↔	mouse	E8–E18	0.3, 0.6, 0.8	IP	10
<i>Anxiety</i>					
<i>Elevated plus maze</i>					
↓	mouse (F)	E15–P12	25	DW to dam	36
↔	rat (M)	E11–E21	12	IG	11
	rat	E14–P7	10	OMP to dam	39
	mouse	E0–P21	7.5	IG to dam	35
	mouse	P4–P21	10	IP to pup	44, 45
	mouse	E8–E18	0.3, 0.6, 0.8	IP	10
<i>Open field</i>					
↑	mouse	E8–E18	0.3, 0.6, 0.8	IP	10
↔	mouse	P4–P21	10	IP to pup	37, 40, 45
	mouse (F)	E15–P12	25	DW to dam	36
<i>Novelty-suppressed feeding/drinking</i>					
↑	rat	E11–E21	12	IG	11
	mouse	P4–P21	10	IP to pup	41, 42
↔	rat	E0–P21	5	IG to dam	47
<i>Defensive withdrawal test</i>					
↔	rat	E12–birth	8, 11, 12	OMP	42
<i>Light-dark box</i>					
↔	mouse (M)	P4–P21	10	IP to pup	37
	rat	P2–P21	17.2	DW to dam	43
<i>Exploration</i>					
↓	rat	E14–P7	10	OMP to dam	39
	rat	E6–E20	12	IG	23
	mouse	P4–P21	10	IP to pup	37, 40, 44, 45
↔	rat	E7–E20	1, 5, 12	IG	19
	rat	P2–P21	17.2	DW to dam	43
	rat	E21–P21	10	oral to dam	53
	rat (M)	E11–E21	12	IG	11
	rat (M)	E13–E20	10	SQ	24
	mouse	E0–P21	7.5	IG to dam	35
	mouse (F)	E15–P12	25	DW to dam	36
	mouse	E8–E18	0.3, 0.6, 0.8	IP	10
<i>Depressive behaviour</i>					
↑	mouse (F)	E0–P21	7.5	IG to dam	35
↓	mouse (M)	P4–P21	10	IP to pup	37
	mouse (F)	E15–P12	25	DW to dam	36
↔	rat (M)	E11–E21	12	IG	11
	mouse	E0–P21	7.5	IG to dam	35
<i>Prepulse inhibition of acoustic startle</i>					
↔	rat	E7–E20	1, 5, 12	IG	19
	mouse (F)	E15–P12	25	DW to dam	36
	guinea pig	pregnancy	7	OMP	54
<i>Motor skill</i>					
↔	rat	E12–birth	8, 11, 12	OMP	42
	mouse (F)	E15–P12	25	DW to dam	36
<i>Thermal threshold</i>					
↔	rat	E21–P21	10	oral to dam	53
	mouse	E0–P21	7.5	IG to dam	35
<i>Postoperative pain sensitivity</i>					
↑	rat	E21–P21	10	oral to dam	53
<i>Social behaviour</i>					
↓	rat	P8–P21	10	SQ to pup	52
↔	rat (M)	E11–E21	12	IG	11
<i>Sexual behaviour</i>					
<i>Sexual motivation</i>					
↓	mouse (M)	E0–P21	7.5	IG	57
↔	rat (M)	E0–P21	7.5	IG to dam	51
<i>Copulatory behaviours</i>					
↓	rat (M)	P8–P21	10	SQ to pup	52
	rat (M)	P1–P21	5	OMP to dam	26
↑	rat (F)	P1–P21	5	OMP to dam	58
↔	rat (M)	E11–E21	12	IG	11
	rat (M)	E13–E20	10	SQ	24
	rat (M)	E0–P21	7.5	IG to dam	51
<i>Aggressive behaviour</i>					
↑	rat (?)	E13–E21	10	IP	61
↔	mouse (M)	E0–P21	7.5	IG to dam	35
<i>Reward</i>					
<i>Reinforcement by cocaine</i>					
↑	rat	E14–P7	10	OMP to dam	39
<i>Spatial memory</i>					
↔	mouse (F)	E15–P12	25	DW to dam	36
<i>Fear memory</i>					
↑	mouse	P4–P21	10	IP to pup	44, 45
↔	rat (M)	E13–E20	10	SQ	24
<i>Circadian behaviour</i>					
<i>Phase-advance to light (late subjective night)</i>					
↑	mouse (M)	E15–P12	25	DW to dam	55
<i>Free-running period</i>					
↓	mouse (M)	E15–P12	25	DW to dam	55

Arrows indicate direction of change; ↔ = no change. IP = Intraperitoneal; SQ = subcutaneous; DW = drinking water; OMP = osmotic mini-pump; IG = intragastric gavage; E = embryonic day; P = postnatal day.

posed to a higher dose of FLX during the early postnatal period were quicker to fall off the cylinder than controls, though this difference was abolished after several training sessions. Though these reports appear contradictory, it is possible that both are accurate; it could be the case that FLX is beneficial to motor coordination during the prenatal period but deleterious postnatally, or that FLX improves motor coordination at lower levels of exposure while impairing it at higher levels.

There is limited evidence that FLX may also affect sensory capability. Lee [50] found that rats briefly exposed to FLX during the early postnatal period crossed shorter distances in the gap-crossing test, which is suggestive of impaired vibrissal function. Though this test has some issues in that it is potentially confounded by motivation or anxiety, the same rats also showed reduced thermal sensitivity in the hot-plate test [50], which likely cannot be explained by such factors. However, Lisboa et al. [35] showed that female and male mice exposed throughout pregnancy and lactation were not different from controls in terms of thermal sensitivity [35], and Knaepen et al. [53] observed no effect of postnatal FLX exposure on sensitivity to thermal (or mechanical) stimuli in male rats, though the treatments used in both studies likely resulted in a substantially lower concentration of FLX than the one used by Lee. Thermal sensitivity was also assessed in a study by Vartazarmian et al. [54], in which male and female guinea pigs were exposed to FLX throughout the entirety of gestation by implanting osmotic mini-pumps into the pregnant sow. Intriguingly, FLX exposure did not alter thermal sensitivity relative to untreated controls, but it did normalize thermal sensitivity by reversing the sensitizing effect of vehicle treatment. The fact that vehicle treatment increased thermal sensitivity, perhaps through an effect of maternal stress associated with repeated mini-pump implantation, is very interesting, and highlights the importance of considering the way in which FLX treatment might interact with the stress caused by most administration methods. Additionally, it was found that, relative to the performance of vehicle-treated animals, FLX did not affect sensorimotor gating function, as assessed by prepulse inhibition of the acoustic startle response, though both vehicle and FLX treatment increased prepulse inhibition relative to untreated controls [54]. In another examination of prepulse inhibition, no effect of FLX treatment was observed in female mice [36], nor has the magnitude of the acoustic startle response itself been found to be altered in guinea pigs [54], female mice [36], or adolescent and adult rats [19].

To date, little work has been done to examine how developmental FLX exposure might affect the circadian system, though some evidence suggests that it is indeed altered. Specifically, exposure resulted in larger phase advances in response to a light pulse, a shortened free-running circadian period in constant darkness, and a decrease in the phase-shifting response to the 5-hydroxytryptamine 1A/7 (5-HT_{1A/7}) receptor agonist 8-hydroxy-2-dipropylaminotetralin [55].

Social, Sexual, and Aggressive Behaviours

In contrast with the emotional and motor behaviours described above, social and reproductive behaviours have been relatively poorly characterized. This is concerning, considering a recent report demonstrating that children with autism spectrum disorders, which are often associated with significant social impairments, were more likely than control children to have been prenatally exposed to SSRIs [56]. Though few studies have examined whether early FLX exposure affects social behaviours in rodents, the findings of those that have suggest these behaviours may be impaired. Aspects of social play are reduced considerably in male, but not female, adolescent rats following postnatal exposure to FLX [52]. Similar effects on the social play of male adolescent rats have also been observed following prenatal exposure [11], suggesting that this effect is consistent regardless of the period of exposure. In adults, a test of preference for investigating a novel conspecific versus an inanimate object showed that adult male and female FLX-exposed rats had less of a preference for the conspecific [52], which could reflect reduced social motivation. Sexual behaviours in male rats are disrupted or decreased by postnatal FLX, administered either directly to the pup or indirectly via the dam's milk [26, 52], but not following exposure throughout pregnancy and lactation in mice, though this treatment was found to reduce sexual motivation, as assessed by preference for investigating a receptive female over an adult male [57]. Other studies have found no effect on male sexual behaviour or motivation following prenatal or combined prenatal and postnatal exposure. Unlike in males, sexual behaviours in female rats are increased following postnatal exposure to FLX [58] (table 3).

Aggressive behaviour represents a particularly interesting case, given that an overabundance of serotonin in humans, caused by loss of the monoamine oxidase A (MAOA) gene, has been found to produce a notable syndrome of impulsive aggression [59], an effect that is mirrored in MAOA knockout mice [60]. This might also be the case following developmental FLX exposure. In one

study, prenatal exposure increased the incidence of fighting bouts in a test of foot shock-induced aggression [61]. On the other hand, Lisboa et al. [35] found that treating dams with FLX throughout pregnancy and lactation exerted no effect on the aggressive behaviour of offspring in the resident-intruder test, other than a non-significant tendency toward increasing latency to the first attack, which could indicate a subtle decrease in aggression. The fact that aggression is not a homogeneous behaviour, and that one study examined aggression resulting from exposure to painful stimuli whereas the other assessed territorial aggression, may account for the difference in findings.

Learning, Memory, and Reward

Limited evidence suggests that developmental FLX exposure may actually improve spatial memory, as decreased escape times have been noted in male adolescent rats in a water maze [23]. On the other hand, the same was not found in female mice [36] or by another group examining the performance of adolescent rats in a water maze and on a test of spontaneous alternation using a very similar dosage and period of administration [19].

FLX exposure during development may also strengthen fear memory in later life, though here, too, the evidence is mixed. In a passive avoidance test, prenatally exposed male and female adolescent rats took longer to enter a compartment previously associated with a shock [23], though a similar study did not find the same effect in either sex [19]. In adulthood, prenatally FLX-exposed male rats showed increased fear memory in a shock-induced place aversion test [11]. On the other hand, postnatally exposed mice have been found to take longer to escape a foot shock in an active avoidance test [44, 45], though this may reflect an altered stress response [44] rather than an impairment in fear learning. Finally, prenatal FLX exposure in rats has been found to increase place preference in response to cocaine administration and increase the number of attempted self-administrations during an extinction trial, indicating that FLX exposure might increase sensitivity to the rewarding effect of this drug [39].

Neuroanatomical Outcomes

As one would expect, given its ability to manipulate behavioural outcomes, FLX has been tied to long-term chemical and physiological changes in the brains of animals exposed in early development. Not surprisingly,

many of these changes are of the serotonin system itself. It is also not surprising that the pattern of findings is complex, mirroring the way in which the behavioural outcomes of FLX exposure differ extensively based on when exposure occurs and when outcomes are assessed. Prenatal exposure reduced serotonin in the frontal cortex of adolescent rats [62], but the same measure was increased in adults and adolescents when exposure was postnatal [43]. Similarly, in adolescents, an increase in serotonin was noted in the ventral, though not dorsal, hippocampus when administration was postnatal [43], yet no effect was found on hippocampal serotonin, or on serotonin in the hypothalamus, striatum, or midbrain, when FLX was given prenatally [62]. By adulthood, however, a decrease in midbrain serotonin had emerged, while frontal cortex serotonin had normalized [62]. This decrease in midbrain serotonin could be related to changes in serotonergic neurons themselves, as postnatal FLX exposure has been found to reduce the number and size of neurons in the raphe nuclei [63], which could also account for the noted reduction in serotonin transporter (SERT) in this region following prenatal exposure [10, 39].

Changes in SERT and serotonin receptor density have been found to show similarly complex patterns. In adolescent rats exposed prenatally, SERT density is decreased in the dorsomedial hypothalamus, but increased in the lateral hypothalamus, hippocampus, and amygdala [64], demonstrating that even regional changes might be obscured by differential changes in subregions. Limited evidence suggests that there is little effect on serotonin receptors at this stage, as no differences were found in hypothalamic 5-HT_{2A} and 5-HT_{2C} density [32] or prefrontal 5-HT_{1A} and 5-HT_{2A} mRNA expression [43]. In contrast, by adulthood changes in SERT density outside the raphe nuclei appear negligible [39, 42, 62, 64] (online suppl. table 2), whereas changes in receptor density emerge, at least in the case of 5-HT_{2A} and 5-HT_{2C} receptors within the hypothalamus, which are downregulated [32]. It will be interesting to see whether this tentative pattern is upheld by future research.

Beyond the serotonergic system, early FLX exposure alters neuronal morphology in regions widely dispersed throughout the brain. For the most part, FLX exposure appears to reduce neuronal complexity later in life. In adolescent rats, early postnatal exposure decreases the dendritic complexity of both thalamocortical afferents and spiny stellate neurons in layer IV of the somatosensory cortex [50], and prenatal exposure reduces the complexity of layer II/III pyramidal neurons in the same region in adolescent and adult mice [46]. This loss of complexity in

the somatosensory cortex may account for the deficits in tactile sensation described above. Similar decreases in either spine density or dendritic complexity have also been noted in pyramidal neurons in layer V of the motor cortex and medium spiny neurons in the striatum [41], though a rare increase in spine density was observed in hippocampal CA1 dendrites in adult mice following postnatal exposure [40].

FLX exposure also appears to have effects on broader systems, causing alterations in neurogenesis and the stress response. Postnatal exposure decreased cell proliferation in the hippocampal dentate gyrus in adolescence, though the number of migrating cells was not altered [38], suggesting an increase in the survival of proliferating cells. Decreased glucocorticoid receptor density and glucocorticoid receptor interacting protein expression have been noted in male, but not female, adolescent rats in the CA3 region, though not in CA1 or dentate gyrus [65]. The effects of FLX exposure on stress responsivity also extend beyond the hippocampus, as decreased serum corticosterone and a decrease in the free corticosterone index have also been noted, again only in males [65]. Additionally, FLX exposure appears to alter activation of certain regions of the brain in response to restraint stress, with adolescent males showing less activation of the basolateral amygdala, and adult males showing less activation of both the basolateral and medial regions of the amygdala [47]. None of these changes were observed in females, nor was activity in the central nucleus of the amygdala or the paraventricular nucleus altered in either sex. Such sex-specific changes might hint at the cause behind the sex differences observed in certain behavioural effects of FLX exposure. Further relating to sex differences, FLX exposure may have effects on certain sexually dimorphic regions of the brain. This has not been well studied to date, but recently it has been reported that postnatal exposure to FLX in male (but not female) rats decreases the volume of the sexually dimorphic nucleus of the preoptic area in the hypothalamus, which is normally larger in males than in females, though it was not found to alter two other dimorphic regions of the brain, the posterior bed nucleus of the stria terminalis and the anteroventral periventricular nucleus [26, 58]. Finally, serotonin may not be the only neurotransmitter system affected by developmental exposure to FLX. One study has reported that exposure during pregnancy and the pre-weaning period reduces the intensity of dopamine-mediated behaviours following pharmacological challenge in female adolescent mice [66]. This raises the possibility of long-term changes in the dopaminergic system, though

more specific analysis is still required. So far, no other changes have been found, though only basal dopamine and norepinephrine levels [62] as well as norepinephrine transporter density in the frontal cortex [42] have been examined.

FLX Exposure and Maternal Stress

Up to this point in the review, the studies discussed have primarily sought to examine the effects of early FLX exposure in the offspring of healthy animals. This approach is certainly of use, particularly for the purpose of isolating the effects of FLX exposure from the effects of maternal stress or depression, which is not easily done in clinical studies. It is the case, however, that people are not given FLX or other antidepressant medications arbitrarily, but rather because they are experiencing a condition, often depression, which may be associated with high levels of stress. Examining the offspring of healthy animals is then of questionable ethological validity when attempting to draw inferences about exposed humans. Though the degree to which human depression can be meaningfully modelled in rodents is debatable, it thus seems likely that inducing maternal stress would be highly valuable when conducting studies in this area. So far, relatively few studies have done so, though the ones that have seem to indicate that it is a worthy endeavour, as maternal stress and FLX exposure appear to interact in ways that differ from the effects of either in isolation. It has been found that prenatal maternal stress potentiates the stress response, as measured by plasma corticosterone, of the offspring when assessed during adolescence, and that postnatal FLX treatment reverses this effect while having no effect in the absence of stress [67]. Moreover, when adult offspring were assessed, a similar pattern was observed in hippocampal dendritic spine and synapse density, with stress causing a decrease and FLX reversing it, as well as behaviourally in the Morris water task, with prenatal stress causing a decline in spatial and reversal learning and postnatal FLX reverting these functions to normal [67]. Postnatal FLX has also been found to reverse some other behavioural alterations caused by prenatal maternal stress, including depression-like and anxiety-like behaviours in adolescent rats [38, 68], and to offset the effects of prenatal maternal stress on postoperative pain. Specifically, stress dulled the hypersensitivity to mechanical stimuli induced by hindpaw incision, whereas postnatal FLX exposure intensified this postoperative pain [53]. In combination, the effects appeared to cancel out, leaving

offspring no different from normal. In the hippocampus, postnatal FLX normalizes the prenatal stress-induced downregulation of neurogenesis [38]. However, when administered in the absence of stress, postnatal FLX appears to cause a decrease in hippocampal proliferation, though not in the number of migrating neuroblasts, providing another example of how FLX exposure interacts with maternal stress. Though the exact mechanism by which this interaction occurs is not known, it may have to do with the long-term effects of developmental FLX exposure and maternal stress on serotonin levels. It has been observed that FLX exposure decreases serotonin levels in the cortex of adolescent rats [62] whereas prenatal maternal stress increases them [69]. Conversely, it has also been reported that prenatal stress decreases serotonin in mice when measured around the time of weaning, while postnatal FLX treatment alone or in combination with prenatal stress increases serotonin [67]. Thus, while the effects of developmental stress and FLX exposure on the serotonergic system are not entirely clear, it appears that these effects may act in opposition, which could account for why the occurrence of both in tandem normalizes the effects of either in isolation.

Methodological Considerations

In reviewing the literature, it quickly becomes apparent that methodology represents a source of considerable variance between studies of developmental FLX exposure. To begin, dosages vary widely, ranging from 0.3 mg/kg/day [10] to 25 mg/kg/day [36, 55]. Furthermore, choice of dosage is frequently left unjustified, and only a small subset of studies has assessed the concentration of FLX and NFLX present in the blood or brain of the offspring. In order to ensure that studies modelling developmental FLX exposure in non-human animals are relevant to the clinical situation, as well as to allow comparisons between studies, it is essential to establish the levels of FLX and NFLX that are produced by various protocols of exposure, and to compare these numbers to those observed in human mothers and infants. Some studies have taken initial steps toward this goal. Capello et al. [42] identified the dosage of FLX that produces serum levels in rat dams that are similar to the levels observed in pregnant women. Other studies have also attempted to mimic the FLX levels of post-partum women in dams [65, 70]. Though this represents a good start, more studies should take similar steps. Going a step further and reporting changes in serotonin levels in the off-

spring during and following FLX exposure would also be very helpful, though it is important to note that global serotonin levels may not tell the whole story, as they may not reflect regional increases or decreases, changes in the intracellular-extracellular balance, or changes in receptor density and function.

In addition to dosage, route of administration is also important to consider, as it will impact the pharmacokinetics of the drug. To provide one example, a dose given orally will be subject to first-pass metabolism by cytochrome P450 enzymes in the liver, which are more active during pregnancy [71], and as such will result in a lower concentration than a dose administered by intraperitoneal injection or outside the pregnant state. Further complicating the issue, cytochrome P450 is inhibited by FLX, producing a non-linear relationship between drug dosage and drug concentration [72]. Additionally, stress is induced by some methods of administration, such as gavage [73] and injection [74], whereas other methods, such as administering FLX in the drinking water, are non-intrusive. If the goal is to study the effects of FLX in isolation from stress, then such stressful methods represent a potential confounding factor. On the other hand, the addition of stress may increase a study's ethological validity, as discussed above. It is also important to consider differences in pharmacokinetics between species, and how this might impact the choice of administration method, especially when attempting to model human exposure. For example, because the half-lives of FLX and NFLX in mice (6 and 12.3 h, respectively [75]) are drastically shorter than in humans (1–3 and 7–15 days, respectively [75]), continuous administration of FLX, by osmotic minipump or through the drinking water, can stabilize drug levels in the blood to better mimic the clinical situation than methods that provide a single, daily bolus, such as injection or gavage [42].

Special considerations arise when the source of FLX is maternal. First is the potentially confounding effect of altered maternal behaviour. Though seldom examined, it does indeed appear that FLX treatment can alter the behaviour of rat dams. It has been reported to increase the amount of licking and grooming the dam performs on the pups [76] as well as the incidence of nursing from the arched-back posture [70], in which the dam is more active and engages in more licking/grooming. This is an important finding, as increases in licking/grooming and arched-back nursing are associated with long-term decreases in the physiological response to stress, as well as decreases in anxiety-related behaviours [for a review, see 77]. Thus, there exists a mechanism by which maternal

FLX treatment may confound examination of the direct effects of FLX exposure on the offspring. However, other studies have shown that maternal FLX treatment does not alter the total time dams spend licking/grooming or nursing the offspring [38, 65, 70], suggesting that if FLX treatment does affect maternal behaviour in rat dams, it does so subtly, and perhaps in a way that privileges very specific behaviours. If this is the case, then it may not be recommendable for studies to include cursory examinations of maternal behaviour, as such examinations may give the impression that maternal behaviour is not altered when in fact they lack the specificity to detect the relevant effects. A thorough examination of how FLX administration affects the behaviour of mouse dams would also be useful, as such an assessment is presently lacking. Second, litter effects, which result from the use of multiple pups from a given litter within an experimental group [78], can create difficulty in interpreting results. Specifically, litter effects can artificially reduce within-group variability, or cause it to appear that there is a difference between experimental groups that is, in actuality, not due to the treatment but to differences between litters. The latter is particularly relevant to studies in which FLX (or any drug or treatment) is administered to the dam, as all offspring from a single litter will necessarily be exposed to the same treatment. Thus, in such cases it is recommended that researchers use only a single pup per litter, or use multiple pups and control for litter effects during statistical analysis.

Conclusion

Though a considerable amount of research has been done on the topic of developmental FLX exposure, the emerging picture is, overwhelmingly, one of great heterogeneity. This should not be surprising, considering the number of potentially impactful variables that often differ between studies, as well as the myriad roles of serotonin in development [79, 80]. Overall, it is clear that early FLX exposure can induce long-term alterations in the serotonergic system and other areas of the brain, and that these alterations can affect behaviour, in turn. While decreased exploratory locomotion is the most common finding, social and sexual behaviours, as well as anxiety- and depression-like behaviours, also seem to be particularly susceptible. However, the precise effects, and in many cases even the directionality of the effects, are yet unresolved, and many important details, including the neural mechanisms underlying particular behavioural alterations, remain to be delineated.

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