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Asymmetry of turning behavior in rats is modulated by early life stress

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ABSTRACT

Atypical leftward behavioral asymmetries have been associated with early life stress and psychopathologies in humans and animals. Maternal separation (MS) is a frequently used model to investigate early life stress and psychopathologies but has not yet been studied in terms of asymmetries. This study aims to investigate whether prolonged MS induces atypical leftward asymmetries in the turning behavior of rats. MS was performed from postnatal days 2–20 followed by a second stressor from postnatal days 21 − 40. Asymmetry of turning behavior was then examined in the elevated plus-maze test upon weaning (juveniles and dams) or adolescence. The number of left and right turns was calculated per animal using the deep learning software package DeepLabCut enabling markerless pose estimation. Then, a lateralization quotient (LQ) was determined for each animal allowing to investigate the strength as well as the preferred side of asymmetry. LQ analysis revealed a significant leftward asymmetry in the prolonged stress group. Moreover, analyzing the number of turns revealed significantly more left than right turns in total in this group. Control animals showed no asymmetries in turning behavior. These results indicate that prolonged stress during the early postnatal days led to atypical leftward turning behavior. The stress-induced atypical asymmetry might be a mediator of early life stress and the development of psychiatric disorders.

1. Introduction

Hemispheric asymmetries are a general principle of functional organization in the vertebrate brain and have been reported for many behaviors and cognitive systems [1–10]. In both humans and animals, it has been shown that genetic and non-genetic factors are involved in the ontogenesis of hemispheric asymmetries [11–17]. A recent study utilizing the UK Biobank dataset found that in humans, several early life factors significantly contributed to left-handedness [18]. These factors included the year and location of birth, birth weight, being part of multiple births, the season of birth, breastfeeding, and sex.

As both low birth weight and being part of multiple births are potentially linked to birth stress, it is conceivable, that early life stress, e.g. via hormonal effects, could affect the ontogenesis of hemispheric asymmetries. Indeed, it has been shown that in humans and other species, there are relations between different forms of stress (early life stress, chronic stress, and acute stress), but the direction of these relations do not follow a consistent pattern [19]. One problem here might

be that in research in human participants, an experimental variation of early life stress conditions is impossible due to obvious ethical reasons. Therefore, all analyses are usually post-hoc, e.g. by comparing data on early life events from medical records between different individuals and linking them to hemispheric asymmetries as adults. While there are studies on the effects of early life stress and maternal separation (MS) in specific human populations, e.g. in the English and Romanian Adoptees study [20], these works, unfortunately, do not include data on hemispheric asymmetries.

Therefore, research in animal models that induce early life stress in a controlled manner is necessary to investigate the consequences of early life stress on brain asymmetry. One well-established and widely used animal model to investigate the consequences of early life stress is MS [21]. However, as the MS procedure varies across studies, e.g. in the time being separated daily and the number of days MS is conducted, the results can be inconsistent [22,23]. Therefore, an already established MS protocol was used that consists of daily separation for 4 h over the first postnatal weeks (postnatal day 2–20) which leads to increased

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depression-like behavior in adolescent female and male Sprague-Dawley rats [24]. To investigate the consequences of post-weaning chronic stress exposure, social isolation is a frequently used paradigm that is also known to induce anxiety- and depression-like phenotypes [25,26]. Using both stress paradigm allows investigating different stress-sensitive windows (early and late childhood) separately as well as an effect of both stressors experienced consecutively.

Neuronal asymmetries are also well-studied in the rat. Researchers have found hemispheric dopaminergic asymmetries in the medial prefrontal cortex matching the rats turning asymmetry after ethanol injection (right turning rats had activation in the right hemisphere) [27] as well as dominant right prefrontal cortex activation in stress regulation [28]. It is known that rats, as well as other rodents, have an intrinsic side preference and that this preference is modulated by dopamine release [29]. More precisely, rodents turn in the direction contralateral to the striatum side containing more dopamine. This behavior can be modulated by e.g. amphetamine and potentiated into circling behavior [29]. In a genetically modified circling rat, researchers found lower striatal dopamine ipsilateral to the preferred rotation side [30]. Stress exposure led to an increase of dopamine release in the contralateral striatum in modified rats but not in wildtype rats [30]. Neonatal novelty exposure using the novel cage test during the first 3 weeks of life led to a developmental stable turning bias [31]. Turning behavior was defined as 90° rotation and was analyzed during a 5 min novel cage test. Then, the lateralization score was calculated showing a right-shift in turning bias in the novel cage in males but not females [31]. However, most studies investigating asymmetry behavior in rats find favor of the right side e.g. when testing turning behavior in mazes [32]. Moreover, they show strong lateralization of the individual but no side bias at the population level in head-turning [33] and paw preference (54 % right-sided) [34]. Interestingly, when investigating head-turning asymmetry, left-turning rats showed increased behavioral despair in the forced swim test compared to right-turning rats [33].

As mentioned, asymmetries can also be assessed in behavior. As in humans, there are different behaviors to analyze laterality in rats. Atypical lateralization in rodents can be investigated by analyzing e.g. paw preference [34], head-turning [33], or general body turning behavior [27,30–32]. The benefit in analyzing general turning behavior in a setup where the animal can move freely is that turning behavior occurs more naturally. Therefore, when analyzing turning behavior in the rat, several behavioral tests such as the open field, the T-maze, and the elevated plus maze have proven to be very useful as animals have to turn frequently to navigate through the maze [32].

A recently published review investigated the role of stress in psychiatric disorders and atypical lateralization [35]. It was concluded, that early life stress as well as chronic hypothalamic-pituitary-adrenal (HPA)-axis elevation change structural and functional asymmetries [35]. More precisely, an acute or chronic increase in glucocorticoids impacts the corpus callosum (which is important for interhemispheric communication) leading to altered hemispheric asymmetries [35]. Thus, an increased stress exposure would lead to increased glucocorticoid levels, and consequently to more atypical asymmetries. These atypical asymmetries were also observed in psychiatric patients [35].

As mentioned above, hemispheric asymmetries are especially vulnerable to early life stress. Exposing rats to chronic stress early in life should shift the hemispheric asymmetry compared to controls. This shift in hemispheric asymmetry can then be observed in altered turning behavior. To investigate whether different times of early life stress exposure have a different impact on asymmetric turning behavior, animals were either exposed to MS in the early postnatal days or to isolation in late childhood. For the consequences of prolonged chronic stress exposure, one group of animals was subjected to MS followed by isolation. Consequences should be most obvious in this group.

Besides modulation via stress exposure, some behavioral asymmetries have also been found to be hereditary in humans [36,37]. Studies in inbred mice did show heritability of paw preference regarding

strength but not direction of preference [38,39]. However, as not all behavioral asymmetries are inherited, another explanation for this transgenerational modulation of asymmetry might be via epigenetic mechanisms [17]. This is particularly interesting regarding the effects of maternal (or intrauterine) stress exposure on offspring asymmetries [17]. It is also possible, that some asymmetries are controlled by genetic and others by environmental factors [36,37].

In this study, rats were exposed to MS, isolation, or both and compared to controls. Turning behavior was analyzed during a 5 min elevated plus maze test to investigate turning asymmetries after stress exposure. Moreover, dams were analyzed as well, allowing for a mother-offspring regression analysis to investigate whether maternal turning behavior could predict offspring turning behavior. To our knowledge, this is the first study to investigate asymmetries in turning behavior in an animal model for MS and isolation.

2. Methods

2.1. Animals

Animals were housed under standard conditions (22 ± 2 °C room temperature, 55 ± 25 % humidity) and standard lighting (12 h/12 h) with free access to water and food. Experiments were conducted under the principles of Germany's Animal Welfare Act after approval by the LANUV (Landesamt für Natur, Umwelt und Verbraucherschutz Northrhine-Westfalia). 16 timed-pregnant Sprague-Dawley rats (Charles River Laboratories, Sulzfeld, Germany) were single housed upon arrival and were divided into the MS ($N = 8$) or control group (CG, $N = 8$). Postnatal day (PND) 0 was equal to the day of birth of the pups. At PND2 pups were sexed and culled to 10, if possible five male and five female pups, leading to a total of 79 female and 78 male pups.

2.2. Stress induction

In total, animals were assigned to 4 different groups. The MS group, the CG, the isolation stress (IS) group, and both MS and IS (MSIS) group. Besides stress exposure, 3 different age groups tested emerge as follows: dams, juvenile rats (PND21), and adolescent rats (PND41).

MS was carried out as previously described [24]. In brief, pups were separated from their mother and siblings from PND2 – PND20 for 4 h every day during the dark (= active) phase. Pups were placed in separate cages (for the early days of separation, pups were placed on a heating mat adjusted to 37 °C, pups were divided by a self-made plastic grid which allowed placing home cage bedding on top of the heating mat). At PND21 all pups were weaned. One animal per litter and sex was tested in the elevated plus maze on the day of weaning as well as the dams. The littermates were either group-housed (GC) or single-housed (isolation stress; IS) until PND41. All animals were then tested in the elevated plus maze. Single housing is considered a stressor. Therewith, animals in the IS group, that were not subjected to MS before, received a late stressor instead. Animals in the IS group, that were already exposed to MS, were exposed to a second stressor (MSIS). Animals placed in group-housing were either only subjected to MS or received no stress at all (GC). Therewith, the different groups are composed as follows.

Mothers

- CG: $N = 8$
- MS: $N = 8$

Offspring PND21:

- CG: $N = 8$ females and 8 males
- MS: $N = 8$ females and 8 males

Offspring PND41:

- CG: N = 24 females and 24 males
- MS: N = 23 females and 22 males
- IS: N = 8 females and 8 males
- MSIS: N = 8 females and 8 males

2.3. Analysis of turning behavior asymmetries

Behavioral testing was carried out in the dark phase under red light. The elevated plus maze consists of two open arms (50cm × 10cm) and two closed arms (50cm × 10cm) and a center connecting the arms. The maze is adjusted at 50 cm in height. Animals were placed on the elevated plus maze facing a closed arm and could discover the maze freely for 5 min while being filmed using an HD Webcam (C920 Pro, Logitech) connected to a laptop. A video-based offline tracking was performed via python software 'DeepLabCut', [40]. Horizontal x–y coordinates of the head, the body center, and the tail base were extracted and smoothed with a median filter to remove noise. Body and head orientation were determined as vectors from tail to body, and from the body to head, respectively. Turning behavior was defined as turning the head more than 45 degrees from the body center which was then counted across a session.

2.4. Statistical analysis

Dams and offspring were analyzed separately, as stress exposure was either early in life (offspring) or in the sensitive postpartum time (dams). Data in dams were analyzed using 2 × 2 repeated-measures ANOVA with the within-subjects' factors side (left turning, right turning) and the between-subjects' factors group (CG, MS). Data in offspring was analyzed using 2 × 4 repeated-measures ANOVA with the within-subjects' factors side (left turning, right turning) and the between-subjects' factors group (CG, MS, IS, MSIS). Post-hoc test was corrected for multiple comparisons by using Bonferroni correction. The effective alpha-level to reach significance was adjusted for each test regarding the number of interactions applied. Additionally, a lateralization quotient (LQ) was determined for each animal following the formula LQ = ((R−L) / (R + L)) ×100, with R indicating the number of right turns and L indicating the number of left turns. The LQ ranges between -100 and 100, with positive values representing a right-sided turning bias and negative values indicating a left-sided turning bias. Moreover, we also assessed individual side preferences independent of the strength of lateralization by classifying animals as being right-preferent (positive LQ) or left-preferent (negative LQ).

In order to be able to estimate heritability, LQ data from mothers were used to predict LQ data from offspring using linear regressions. Heritability (h^2) in this model is equivalent to the regression coefficient b of the parent-offspring regression [41]. Statistical analysis was performed using SPSS (IBM SPSS Statistics 26).

3. Results

3.1. Asymmetries in dams

Since the overall N was low in dams (N = 16) we tested the data for normal distribution using KS tests in order to decide whether parametric testing would be possible. Both, the amount of left-sided (p = .98) and right-sided turning behavior (p = 1.00) did not significantly deviate from normal distribution. Therefore, we used a 2 × 2 repeated-measures ANOVA with the within-subjects' factors side (left turning, right turning) and the between-subjects' factors group (CG, MS) to investigate the data in dams. Both, the main effect of side ($F_{(1,14)}$ = 0.42; p = .53) and the main effect of group ($F_{(1,14)}$ = 0.01; p = .95) failed to reach significance. The interaction side × group approached significance ($F_{(1,14)}$ = 3.56; p = .08) and tentatively suggested that animals in the MS condition showed a more rightward asymmetry (number of left turns: 23.88 +/- 7.77 SD; number of right turns: 30.50 +/- 7.29)
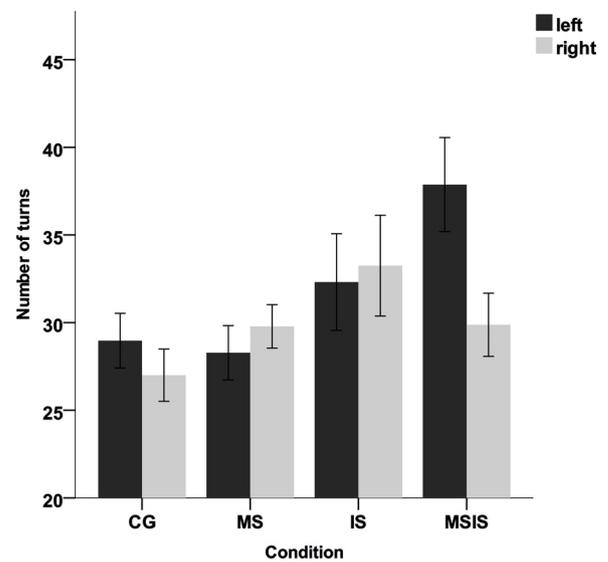
Fig. 1. Distribution of left turns and right turns in the four offspring groups. Mean number of left (black) and right (grey) turns per group ± SD is shown. MSIS animals show significantly more left turns (37.88 +/- 10.73) than right turns (29.88 +/- 7.21).

than animals in the CG (number of left turns: 28.62 +/- 8.86; number of right turns: 25.38 +/- 5.93). However, since the effect failed to reach significance, this relation is at best weak.

3.2. Asymmetries in offspring

Fig. 1 shows the distribution of left turns and right turns in the four offspring groups. We conducted a 2 × 4 repeated-measures ANOVA with the within-subjects' factors side (left turning, right turning) and the between-subjects' factors group (CG, MS, IS, MSIS) to investigate the data in offspring. Both, the main effect of side ($F_{(1,152)}$ = 2.46; p = .11) and the main effect of group ($F_{(1,153)}$ = 2.30; p = .08) failed to reach significance. However, the interaction side × group reached significance ($F_{(3,153)}$ = 2.93; p = .036; partial η^2 = 0.05). To further investigate this effect, we conducted Bonferroni-corrected post-hoc tests.

Post-hoc tests failed to reach significance for the CG (number of left turns: 28.97 +/- 12.47; number of right turns: 27.00 +/- 11.94; p = .20), MS group (number of left turns: 28.28 +/- 12.10; number of right turns: 29.79 +/- 9.69; p = .33), and IS group (number of left turns: 32.31 +/- 11.04; number of right turns: 33.25 +/- 11.50; p = .76). In contrast, the post-hoc test reached significance for the MSIS group (p = .009), indicating significantly more left turns (37.88 +/- 10.73) than right turns (29.88 +/- 7.21) in this group.

To further analyze this effect independently of individual reaction rates, we calculated one-sample t-tests against zero for the LQ in all four conditions (see Fig. 2). This effect failed to reach significance for the CG ($t_{(63)}$ = -1.24; p = .22), the MS group ($t_{(60)}$ = 1.51; p = .14), and the IS group ($t_{(15)}$ = 0.17; p = .87). However, in the MSIS condition, there was a significant leftward asymmetry (LQ = -11.28 +/- 15.89; $t_{(15)}$ = -2.84; p = .012).

In order to test whether there were any differences in the side of preferences independent of the strength of lateralization, we classified animals as being right-preferent (positive LQ) or left-preferent (negative LQ) and used a non-parametric Kruskal-Wallis test to determine differences in the distribution of these categories between the four groups. The effect reached significance ($\chi^2_{(3)}$ = 12.23, p = .007), indicating that the four groups showed different distributions of left- and right-preferent animals (see Table 1).

We then compared the groups directly with each other using Mann-
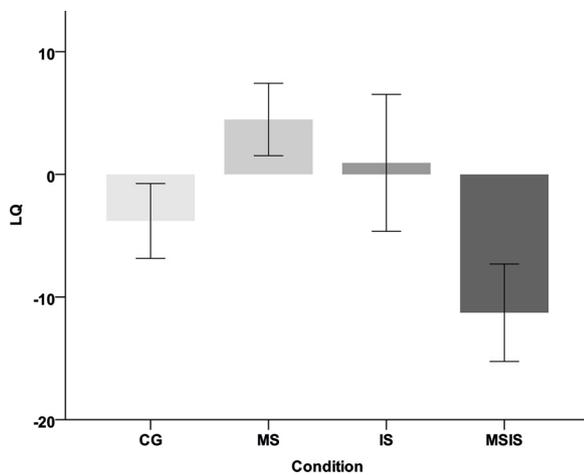
Fig. 2. Lateralization quotient (LQ) in the four offspring groups. Positive values represent a right-sided turning bias and negative values indicate a left-sided turning bias. MSIS animals show a significant leftward asymmetry (LQ = -11.28 +/- 15.89; $t_{(15)} = -2.84$; $p = .012$).

Table 1
Number of left-preferent and right-preferent animals in the four offspring groups. GG: Control group, MS: Maternal separation, IS: Isolation stress, MSIS: MS + IS.

		Left side	Right side	
Group	**CG**	38	26	64
	MS	23	38	61
	IS	7	9	16
	MSIS	13	3	16
Overall		81	76	157

Whitney U-tests. Here it was shown that the MS group had a significantly more rightward distribution than the CG (U = 1529, p = .016). The other two stress groups did not differ from the CG (all p's > 0.10). Moreover, the MSIS group had a significantly more leftward distribution than the MS group (U = 275, p = .002).

In the last step, we used an univariate ANOVA with absolute LQ's in order to test whether there were any differences in the strength of preferences independent of the side of lateralization. However, the effect failed to reach significance ($F_{(3,153)} = 0.61$; $p = .61$).

3.3. Heritability

In order to investigate, whether hemispheric asymmetries in mothers predicted hemispheric asymmetries in offspring, we used LQ parent-offspring regressions (see Table 2). Parent-offspring regressions failed to reach significance in all groups (all p's > 0.24) and heritability quotients were generally low (all $h^2 < 0.16$).

Table 2
LQ parent-offspring regressions for the different groups. GG: Control group, EG: Experimental groups, MS: Maternal separation, IS: Isolation stress, MSIS: MS + IS.

Group	R^2	F	h^2/b	T
All offspring	0.008	$F_{(1,155)} = 1.27$; p = 0.26	0.90	T = 1.12; p = 0.26
CG	0.003	$F_{(1,62)} = 1.65$; p = 0.69	0.05	T = 0.41; p = 0.69
All EG	0.004	$F_{(1,91)} = 0.38$; p = 0.54	0.07	T = 0.62; p = 0.52
MS	0.02	$F_{(1,59)} = 1.33$; p = 0.25	0.15	T = 1.15; p = 0.25
IS	0.0003	$F_{(1,14)} = 0.004$; p = 0.95	0.02	T = 0.06; p = 0.95
MSIS	0.04	$F_{(1,14)} = 0.52$; p = 0.48	-0.19	T = -0.72; p = 0.48

4. Discussion

The present study aimed to investigate the effect of early life stress on behavioral asymmetries in a rodent model. Turning asymmetry was investigated after MS, IS, MSIS, or no stress (CG). Only MSIS led to a significant leftward shift in turning asymmetry compared to no turning asymmetry in CG. MS exposure of dams during the post-partum time did not affect turning behavior. However, a rightward trend was observed in MS dams similar to MS offspring. The turning behavior of dams was not predictive of offspring turning behavior.

Most studies in rats found no turning bias on a population level in the control groups even though the individuals are strong and stable lateralized [32–34]. The finding of chronic stress-induced atypical asymmetry is in line with previous findings indicating a shift of behavioral asymmetry to the left side. As shown in human and animal studies, stress exposure often leads to greater activation of the right hemisphere [19] resulting in more leftward behavioral asymmetry. These altered hemispheric asymmetries have repeatedly been associated with psychiatric disorders [42–47]. For example, studies on frontal electroencephalograph (EEG) alpha oscillation asymmetries in schizophrenia patients found consistent relative left-sided resting frontal alpha power in patients instead of equally distributed in healthy humans [47,48]. Greater left-sided resting EEG alpha indicates reduced activity in the left hemisphere [47]. In healthy humans, a greater left frontal activity is associated with positive emotion and approach behavior whereas a greater right frontal activity to negative emotion or avoidance behavior such as withdrawal [49]. Handedness, as a behavioral measurement of asymmetries, has been most extensively studied in schizophrenia. Here, several meta-analyses confirmed a more than 1.5 fold increased odds ratio for non-right-handedness (left-handedness and mixed-handedness) in people diagnosed with schizophrenia compared to healthy controls [46,50]. These studies underline the association of atypical functional and behavioral lateralization and psychiatric disorders. Of note, a study investigating left-handedness and depression in children found a significantly higher prevalence of depression in left-handed children [51]. As in this study, juvenile and adolescent offspring were analyzed, the results in atypical turning behavior are in line with a higher prevalence of depression in left-handed children [51]. The atypical leftward asymmetry found especially in schizophrenia and depression as well as after early life stress might be explained by the valence hypothesis. Regarding that hypothesis, the right-hemisphere is associated with negative emotion and the left hemisphere with positive emotion [52].

Lesion studies in humans revealed different hemispheric functions of emotion processing with the left frontal hemisphere controlling positive emotions triggering approach behavior whereas the right frontal hemisphere is responsible for negative emotions and avoidance behavior [53]. Damage to one hemisphere reduced the emotionality respectively and thus proving hemispheric control of emotion [53]. This emotional lateralization is found across all vertebrates, including domestic and non-domestic animals as well as primates and humans. Emotions as fear/anxiety and aggression always activate the right hemisphere except in fish, probably as fish are not responding emotionally to presented stimuli. The reaction to food reward, on the other hand, is dominated by the left hemisphere across species [54]. For example, chicks and adult hens both show a left eye preference when observing aerial predators [55,56]. The same left-side preference was found in common wall lizards [57,58], in three species of toads [59], in fish [60], and in domestic cattle herds (when observing novel stimuli) [61]. Dogs confronted with a dominant dog (fear-inducing stimuli) show more leftward tail wagging [62]. Lesion studies in mice and rats underline this asymmetric side preference in emotional situations. In mice, a right-hemispherectomy led to an increase in immobility time in the forced swim test [63]. In a study inducing side specific lesions of the medial prefrontal cortex in rats, only right or bilateral lesion reduced stress-induced cortisol levels [64]. To sum up, asymmetric emotion

processing is consistent and stable across animals all showing right-hemispheric control of negative emotions expressed in leftward behavioral asymmetries.

When investigating the human stress response and asymmetric cerebral hemispheres, some researchers suggest that the cerebral stress response, including the HPA axis, is controlled mainly by the right hemisphere [65]. Aversive situations evoke a stress response and consequently negative emotions [66]. Prolonged and unavoidable exposure to aversive situations leads to an adaptation of the organism potentially resulting in passive behavior or learned helplessness. Administering antidepressants reverse this behavioral effect in rats [66]. Thus, experiencing stress leads to negative emotions that trigger avoidance behavior in rats. As emotional processing is asymmetric, a chronic increased right hemisphere activation results in more left-sided behavior [19].

Interestingly, MS or IS alone was not enough to induce significant altered behavioral asymmetries. It might be possible that a high level of chronic stress is needed before an altered asymmetry manifests in behavior. Children with lower birth weight show more lateralized hemispheric blood flow when exposed to acute stress than children with a normal birth weight exposed to acute stress [67]. Acute stress exposure in healthy humans leads to more negative effects when presented with emotional faces [68]. Stressed participants showed a faster response for angry faces when occurring in the left visual hemisphere than non-stressed participants [68]. Both studies indicate that overall higher stress exposure might lead to more lateralized hemispheric functioning. Similar results have been found in patients suffering from posttraumatic stress disorder (PTSD). In a study investigating children suffering from PTSD and handedness compared to healthy controls, PTSD symptom severity significantly correlated positively with mixed laterality [44]. In an EEG study with adult PSTD patients and controls, left-sided frontal activation was associated with less emotionally intense reactions to negative stimuli in PTSD patients [45]. Another study in soldiers revealed that high levels of childhood trauma together with high levels of PTSD symptoms were associated with greater frontal activation asymmetries, depending on symptoms respectively [69]. Recently it has been shown that mice have facial expressions of their emotional state that are associated with neuronal correlates of emotion [70]. More interestingly, the study revealed that emotions depend on an innate or learned value in mice as well and thus are altered by experience or circumstances (e.g. a thirsty mouse has more pleasure when drinking) [70]. Therefore, a situation that is experienced to elicit negative emotions might trigger a stronger negative reaction when reexperienced (second stressor). As rats exposed to MSIS experience two consecutive stressors, their emotional state might be more aversive compared to only MS or IS rats, leading to stronger activation of the right hemisphere.

Elevated levels of steroids, such as glucocorticoids, might influence cerebral asymmetries as well by altering callosal communication [19,71]. Glucocorticoids are important for normal brain maturation [72]. But exposure to elevated glucocorticoid levels of the fetal brain modifies the HPA axis function and stress reaction for a lifetime [73]. Of note, elevated glucocorticoid levels and HPA activity are also associated with depression [74]. Prenatal stress exposure in humans by e.g. maternal factors or birth complications are already known to influence the ontogenesis of behavioral asymmetries [75]. As rats are born prematurely [73] the maturation of the brain in the early postnatal days of rats is comparable to prenatal human brain maturation. Early life stress is also associated with a greater risk to develop psychiatric disorders like depression [76].

Regarding mother-offspring regression, the heritability of LQ's generally was low and parent-offspring regressions for LQ's failed to reach significance in all groups. This is consistent with the existing literature on the heritability of hemispheric asymmetries on the behavioral level in rodents. In studies investigating handedness (paw preference) in mice, no genetic influence could be established [38,39].

Analyzing 3 generations of selected inbred mice for paw preference revealed stable preferences for one paw in different behavioral tasks [38]. Moreover, both left and right paw preferences were observed in a genetically uniform inbred population [38]. By then only mating left-handed female inbred mice with left-handed male mice and right-handed mice respectively, as well as mixed handed mating pairs, researchers were able to investigate the heritability of paw preference in mice [39]. The results show a clear preference for one paw in most mice but no influence of genetic predisposition [39]. Therefore, environmental influence on behavioral lateralization is expected [38,39,75]. So far, epigenetic mechanisms are thought to be a modulation factor in parental influenced lateralization patterns [75]. In humans, however, some behavioral asymmetries such as handedness [37] and cognitive factors [36] show high heritability. Therefore, it seems possible, that some lateralized functions are controlled by genetic factors whereas others might be regulated by environmental influences [36,75].

Our results underline the findings of atypical leftward behavioral asymmetry after stress exposure. In line with previous studies, we found atypical leftward behavior after prolonged chronic stress. Given that early life stress is a risk factor to develop psychiatric disorders, hemispheric asymmetries altered by early life stress might be a mediator for the development of psychiatric disorders as well. More interestingly, hemispheric and behavioral asymmetries might serve as applicable biomarkers for high-risk individuals before clinical symptoms manifest. Of course, more studies are needed to prove its validity for diagnostic use.

In this study, no corticoid levels were measured. Therefore, the analysis of the individual actual stress level was not possible and thus no correlation of turning asymmetry and corticoid level could be made. Moreover, only turning behavior was analyzed. Other lateralized behaviors might be more susceptible to MS or IS. Futures studies should, therefore, investigate the consequences of early stress exposure at several developmental stages until adulthood. Including multiple behavioral tests like paw preferences, turning behavior, and head-turning behavior will allow assessing more behavioral asymmetries. Furthermore, corticoid levels should be assessed. As blood sampling to e.g. measure corticoid levels in animals induce stress as well sampling should occur before testing. Investigating hemispheric asymmetries as well will also allow a more precise insight into neuronal correlates of altered behavioral asymmetries.

5. Conclusion

Chronic stress exposure during early life leads to atypical leftward asymmetry in turning behavior in an animal model of early life stress. Moreover, as the turning behavior of mothers was not predictive for offspring it might be controlled by environmental rather than genetic factors. Analyzing turning behavior after stress proves to be a good model to investigate atypical leftward asymmetries observed in psychiatric patients. Analyzing hemispheric and behavioral asymmetries could serve as applicable biomarkers for high-risk individuals before clinical symptoms manifest. More studies are needed to prove its validity first.

Authors contribution

A.M. designed the study. A.M. performed the animal experiments. H.M. performed the data analysis and was supported by O.S. The manuscript was written by A.M., H.M., S.O. and N.F. All authors approved the manuscript. This manuscript is our original work and it is submitted for first publication.

Declaration of Competing Interest

The authors declare no conflict of interest.

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