

Research report

Neonatal novelty exposure ameliorates anoxia-induced hyperactivity in the open field

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Abstract

We investigated in an animal model of neonatal anoxia whether effects of oxygen deprivation on emotional reactivity can be reversed by neonatal novelty exposure, a behavioral method, involving daily 3 min away from the home cage for the first 3 weeks of life. Male neonates were exposed to either 100% N₂ gas (Anoxia) or room air (Control) for 25 min on postnatal day 1. Within each of the two treatment conditions, one-half of the neonates were further individually exposed to relatively novel non-home cages for 3 min daily during postnatal days 2–21 (Novel: $N_{\text{Anoxia}} = 20$; $N_{\text{Control}} = 16$), while the other half remained in the home cage (Home: $N_{\text{Anoxia}} = 19$; $N_{\text{Control}} = 19$). Emotional reactivity to an open field was evaluated on postnatal day 25 during four 20-s trials. Among home rats, temporal patterns of open-field activity across multiple trials and initial-trial activity significantly differed between the Anoxia and Control rats. In contrast, these differences were eliminated among the Novel rats. These results show that neonatal novelty exposure, an early-stimulation method that has recently been shown to enhance spatial and social memory, adaptive control of stress response, and hippocampal synaptic plasticity, can also eliminate neonatal anoxia-induced changes in emotional reactivity. These findings suggest that brief and repeated, but mild, changes in the postnatal environment may serve to counteract some of the aversive effects induced by neonatal trauma associated with oxygen deprivation.

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1. Introduction

In humans, birth asphyxia, defined as impaired respiratory gas exchange accompanied by the development of acidosis, occurs among 0.2–3% of live term births [19]. The single most consistent marker of an asphyxic event during the prenatal period is the evidence of neonatal encephalopathy, defined by a constellation of neurological signs observed within the first 7 days. Mild symptoms include increased irritability, hyper-excitability, and sympathetic over-reactivity,

while severe symptoms include coma, seizures, autonomic dysfunction, and brain stem dysfunction [19]. When these neurological signs occur as a result of asphyxia, it is often referred to as hypoxic–ischemic encephalopathy. Unfortunately, a majority of the clinical research deals with severe cases of neonatal encephalopathy and the outcome of asphyxiated infants in functional terms, such as socialization and daily living skills, has been insufficiently investigated [19]. This likely reflects a greater urgency in saving lives over the effort in improving quality of lives later on.

One consequence of neonatal asphyxia that is likely to have long-lasting impact is behavioral hyperactivity. In animal models, anoxia/hypoxia has been shown to increase emotional reactivity to a novel environment as indexed by hyperactivity in the open-field test [2,12,26,41,54,64]. Several decades of infant temperament studies revealed that

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an infant's response profile to novelty shortly after birth can be a predictor of how they will react to novel physical and social environments and of their risks for psychopathology, later in life [28–30,51]. The open-field test is the most extensively studied paradigm for capturing such response to novelty in animal models [6,8,15,44]. At the level of neural mechanisms, changes in open-field activity are coupled with changes in the function of the hypothalamic–pituitary–adrenal (HPA) axis [43,50,65–67], which is known to modulate a variety of cognitive and social functions [10,17,18,35,36,38]. By selecting Open-field emotionality as a dependent variable in this study, we hope to capture an essential aspect of asphyxia-induced changes to facilitate future assessment of anoxic effects on other long-term social and cognitive deficits.

While a range of pharmacological and behavioral treatments has been discussed in the literature [3,19,47,48,55], the present study was designed to determine whether neonatal novelty exposure, an early-life stimulation method, is sufficient to reverse an anoxic effect on open-field emotional reactivity in an animal model. One commonly used animal model of perinatal asphyxia is exposing newborn rats to 100% nitrogen gas, referred to as neonatal anoxia [12,21,32,40,53]. The second is hypoxia, which involves reduced oxygen concentration with (e.g. [46]) or without ischemia (e.g. [11,49]). A third method involves submerging pups under water, shortly after caesarian section [4]. To better isolate the cause of potential functional changes, we favored the anoxia model because a single manipulation was involved rather than two, and because other sources of stress were minimized.

The neonatal novelty exposure procedure was designed to deal with some disadvantages inherent in the commonly used neonatal-handling paradigm [13,33]. First, handled rats differ from non-handled rats in three aspects: separation from the dam; exposure to a relatively novel non-home cage; and experimenter contact during these transfers. Consequently, these three factors are confounded with each other, making any potential generalization from animal models to human birth asphyxia difficult. Second, because the litter is a random factor nested within the handled and non-handled conditions, large between-litter variations can potentially mask the handling effect. Finally, unless pups are sampled from a large number of litters, a genetically unbiased sample of the litter population is difficult to achieve. These may explain why some effects of neonatal handling treatment were not consistently reproduced [9].

The neonatal novelty exposure procedure uses a split-litter (within-litter) design, in which half of the litter experiences a new cage while the other half remains in the home cage. The entire litter of pups is separated from the dam for the same duration and touched by the experimenter at the same time. Thus, this procedure allows us to isolate the novelty-exposure component from the components of maternal separation and experimenter handling per se in the original handling method. By using a split-litter (within-litter) design, one also gains an increased statistical power for detecting the novelty expo-

sure effect. Finally, the split-litter design allows for a matching genetic makeup between the novelty exposed and control groups, thus allowing better isolation of environmental contribution to the observed effect.

With the removal of maternal separation and experimenter handling factors, neonatal novelty exposure involves a significant reduction in the differential treatment of the experimental and control groups in comparison to the handling procedure. Across several measures at multiple levels of analysis, it reliably induced similar effects as those produced by neonatal handling [15,16,39,42], including emotional reactivity and spatial memory performance [56,58], basal stress hormone concentration [61], glucocorticoid receptor function [70], and functional brain lateralization [1,57,59,61,62,68]. Furthermore, neonatal novelty exposure has been shown to increase memory retention in an odour discrimination task [56], social-recognition memory [61], social competence (unpublished data), adaptive corticosterone stress response [60], and hippocampal synaptic plasticity [63]. Here we report an elimination of anoxia-induced changes in emotional reactivity among rats that received this 3 min daily exposure to a non-home environment during the first 3 weeks of life.

2. Methods

2.1. Animals

All experimental procedures were in accordance with the Institutional Animal Care and Use Committee at the University of New Mexico. Sixteen days before giving birth, 11 pregnant Long-Evans hooded dams (Charles River, Raleigh, NC) arrived at the vivarium. The dams gave birth to a total of 132 pups within a time window of 24 h. Birth litter size ranged from 8 to 18 pups. Seventy-four male pups born of these dams were included in this study. Prior to weaning, pups were housed with their own dams. On postnatal day 22, pups were weaned and housed individually in plastic cages (51 cm × 25 cm × 22 cm) with a 12 h light/dark cycle (lights on at 7:00 a.m.) and food and water ad libitum.

2.2. Experimental design and procedures

To assess whether the anoxia-induced increase in emotional reactivity can be counteracted by neonatal stimulation, we used a 2 × 2 factorial design in which anoxia treatment and neonatal novelty exposure were two fixed factors. The anoxia treatment was a between-litter factor, and the 11 litters were pseudo-randomly assigned to Anoxia ($N=6$ litters) and Control ($N=5$ litters) conditions. The novelty-exposure treatment was a within-litter factor, with each litter split into two halves pseudo-randomly, one half assigned to the Novel group and the other half to the Home group. The main dependent measures were open-field ambulatory activity and latency to leave the center of the open field. We also report body weight at weaning. On postnatal day 1, anoxia treatment, culling of the litters to similar sizes, and group assignment to Novel and Home groups, and marking of group identity were carried out. These steps were performed for 1 litter at a time in a counter-balanced order between the Anoxia and Control litters.

Neonatal novelty exposure was carried out daily for 3 weeks between postnatal days 2–21, followed by weighing on postnatal day 22 and the open-field test on postnatal day 25.

2.3. Anoxia procedure

On postnatal day 1, after the dam was moved to a holding cage in the same room, pups were transferred to a plastic airtight chamber (25 cm × 20 cm × 13 cm), equipped with an air inlet at one end and an air outlet at the other. Pups were exposed for 25 min to continuous flow of either 100% nitrogen gas or room air (21% O₂/79% N₂) at a flow-rate of 3 liters/min. The temperature inside of the chamber was kept constant at 31 °C by immersing the chamber partially in water warmed by a submersible heater (Whisper, Tetra, VA). Although pups gasped and appeared pale during exposure to nitrogen gas, they all returned to normal color and appeared to breathe normally within 5–10 min after the exposure. In comparison to other studies using similar procedures that reported mortality rates ranging from 4 to 12% [12,22,26,40,41], the present procedure had a mortality rate of zero. This is consistent with the relatively less exposure to 100% nitrogen gas due to several parameter differences between the present and previous studies, including the use of a low flow-rate and the use of a larger anoxia treatment box in the present study.

2.4. Assignment to Novel and Home groups

Litters were culled to 8–10 pups, immediately after the anoxia or air treatment. Female pups were included only to help maintain comparable litter sizes across litters due to limitations in the scope of the study. Relatively homogeneous body sizes were maintained across the remaining pups. After culling, group membership was marked by tattooing [27] the ventral surface of the digits on the hind paws without anesthesia. Because one-sided marking could confound early-stimulation-induced lateralization [1,16,57,59,61,62,68], we marked both the left and right hind paws of the Novel and Home pups, using two different digit combinations: (a) left first and right fifth; and (b) right first and left fifth. The orders and patterns of marking were counterbalanced between the Novel and Home groups. The anoxia procedure, culling, and marking of the Novel versus Home group membership were carried out in a single session in a separate room from the animal housing.

2.5. Neonatal novelty exposure

Neonatal novelty exposure uses a within-litter design [56] and has been shown effective in creating a range of early-stimulation effects [1,57–59,61,62,68,70]. This procedure was performed in the housing room daily between 11 a.m. and 7 p.m. on postnatal days 2–21 by an experimenter blind to the Anoxia versus Control conditions of the litters. After the dam was transferred to a separate holding cage in the same room, the Novel pups were identified via their markings and were individually transferred to their own clean non-home cages (28 cm × 16 cm × 13 cm) lined with fresh sawdust. These non-home cages were not heated because the goal of the present study was to isolate the novelty-exposure component from the maternal separation and handling, not to determine contributions from specific physical dimensions that contribute to the environmental novelty, such as a temperature difference.

After spending 3 min in the new cage, the Novel pups were returned to the home cage in which the Home pups remained. Up to

five fresh novel cages were used for each litter to allow the novelty exposure for individual pups to be staggered with 1-min intervals. This staggering allowed the experimenter to use the time efficiently and typically 8–10 min were needed for each litter. During this transfer, each Novel pup was yoked to a Home pup; each time a Novel pup was picked up during transfer between the home and novel cage, the yoked Home pup was also picked up similarly but placed back to the home cage. This prevents experimenter handling from becoming a factor that confounds the exposure to an unfamiliar environment. The dam was returned to the litter after the Novel pups were returned to the home cage. Unlike the neonatal handling method [14,33], by equalizing the amount of experimenter handling and separation time from the dam, neonatal novelty exposure ensures that any difference between groups was due to neither a difference in the duration of separation from the dam nor to a difference in the amount of experimenter handling received per se.

2.6. Open-field test

On postnatal day 25, rats were tested in an open field (60 cm × 60 cm × 20 cm) over four 20-s trials (T1–4) in a novel room between 11 a.m. and 4 p.m. In the literature, a variety of trial durations, inter-trial intervals, and number of trials have been used. Typically the trial durations were longer than 20 s, lasting at least several minutes. Often the activity level was measured on multiple days and then averaged. It has been our experience that rats of different experimental treatments tend to behave more similarly, the longer they have been in the open field. Therefore, by focusing on initial exploration, we may increase the sensitivity of our activity measure.

Prior to testing, the floor of the field was sprinkled with soiled sawdust collected from a cage of an adult male rat, which may serve to enhance emotional response in the open field. Rats were tested in batches of eight, two from each of the four conditions. The orders within batches were counter-balanced across experimental conditions. At the beginning of each trial, a rat was placed in the center of the field under a cardboard box, slightly larger than the rat's body size. After a 2-s delay, the box was lifted to allow the rat to freely ambulate. During the inter-trial intervals (~5 min), the rat remained in its home cage, which was placed within the testing room. To minimize interference with the rat's behavior, the experimenter remained at the same location in the room during all trials. The field was wiped clean with a paper towel between trials if defecation or urination occurred. Behavior was videotaped by a camera, mounted directly above the open field for offline analysis.

2.7. Data analysis

Several open-field activity measures were initially coded, including: (1) the latency to leave the center square; (2) distance travelled (the number of squares traversed) during ambulation; (3) subsequent time spent in the center square; and (4) rearing. As a majority of the rats (>75%) did not re-enter the center or did not rear, presumably because of the brevity of the trial duration, measures (3) and (4) did not receive further analysis. We, therefore, performed three-way repeated-measures ANOVA (SPSS), with Trial as a within-subject factor, and Anoxia and Novelty conditions as between-subject factors on both the latency and distance measures. When the interaction effects among Novelty, Anoxia, and Trial were significant, additional ANOVA was performed on two subsets of trials, each repre-

senting a different phase of open-field behavior, based on a previous study of open-field behavior, using similar experimental parameters [15,58]. Specifically, we performed ANOVA on the baseline activity during T1 when the test environment was novel. We performed a separate ANOVA on the change in activity between T2 and T4, reflecting a later phase of more steady change after the initial exposure.

Effect sizes were expressed as Cohen's d when the numerator degree of freedom (d.f.)=1, and as f when the d.f.>1. Because multiple pups were used from the same litter, we first tested for a litter effect. As there were no significant litter effects on any of the measures, individual rats were used as units for the analysis. Because the data violated the assumption of sphericity as well as homogeneity of variance, the F -values and d.f.s were decreased accordingly [37]. For the analysis of body weight data, we took into consideration that the birth size of the litter was a major source of variance in each pup's body weight. ANCOVA was used with litter size as a covariate to remove the effect of birth litter size on body weights.

3. Results

In contrast to the majority of open-field studies using longer duration of open-field exposure (i.e., 2–10 min), the present study used an experimental design consisting of four 20-s trials with an inter-trial interval of 5–8 min, designed to reveal potentially subtle treatment effects in temporal patterns of activity across trials. We found a significant Novelty by Anoxia by Trial interaction on the distance measure (number of squares traversed) ($F(2.75, 192) = 2.966$; $p = 0.037$; $f = 0.21$, Fig. 1a and b), indicating that temporal patterns of ambulatory activity across the four trials differed according to the treatment conditions. Among the Home rats, the Anoxia

treatment effect is indicated by the clear separation of the Anoxia and Control curves (Fig. 1a) and a decrease of this Anoxia effect among the Novel rats is indicated by the reduction of the separation between the Anoxia and Control curves (Fig. 1b). Because of the brevity of the trial duration (20 s), the latency and distance measures are expected to be somewhat correlated: the longer the latency to leave the center, the less time would be left for subsequent ambulation, thus the shorter distance would be traversed. This complementary pattern can be clearly seen when comparing Fig. 1a and b and Fig. 1c and d. Although the three-way Novelty by Anoxia by Trial effect on the latency measure did not reach statistical significance ($p = 0.141$), similar to the distance measure, the separation between the Anoxia and Control curves also appeared to be reduced among the Novel rats in comparison to the Home rats (compare Fig. 1c and d).

3.1. Effects of novelty exposure on ambulatory activity across multiple exposures

Given the significant three-way interaction effect on the distance measure, we made the following focused comparisons. Among Home rats (Fig. 1a), activity in Anoxia rats increased from T1 to T2 but decreased from T2 through T4, while activity in Control rats showed slower but steady increase throughout the four trials. This interaction effect between Anoxia and Trial was significant ($F(3, 108) = 5.622$; $p = 0.001$; $f = 0.40$) and was accompanied by a significant main Anoxia effect ($F(1, 36) = 4.470$; $p = 0.041$; $d = 0.70$). In contrast, among Novel rats (Fig. 1b), the Anoxia rats did not show a significantly different temporal pattern from the Control rats ($p > 0.2$). Among the Anoxia rats, Novel rats showed a significantly different temporal pattern from the Home rats,

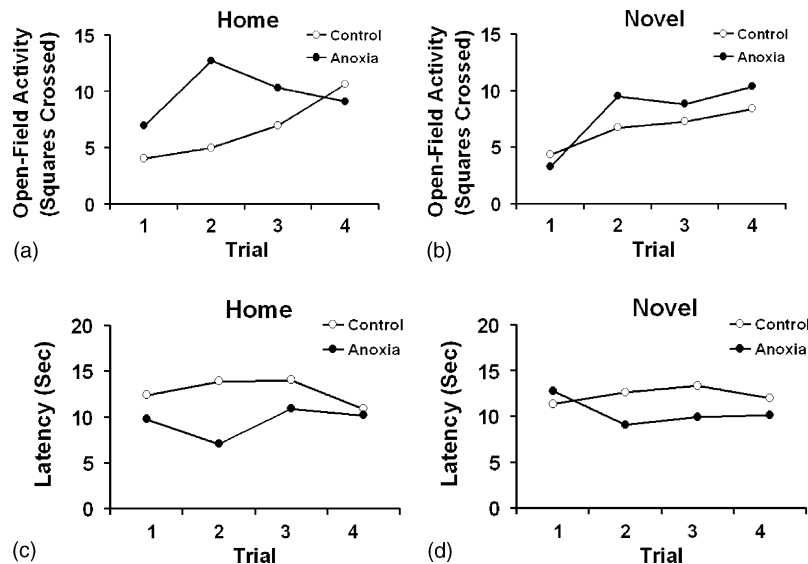


Fig. 1. Effect of neonatal novelty exposure and anoxia on temporal patterns of open-field activity: (a and b), ambulatory activity; (c and d), initial latency to leave the center; (a and c), Home rats: anoxia effect on temporal patterns of open-field activity across multiple trials (T1–T4); (b and d), Novel rats: reduction of anoxia effect by neonatal novelty exposure. Although the Novelty by Anoxia by trial interaction effect was statistically significant only on the distance measure, the qualitative similarity between the distance and latency measures should be noted.

as indicated by a significant Novelty by trial interaction ($F(1, 37) = 4.455$; $p = 0.042$; $d = 0.69$). When the temporal pattern across trials was not considered, no Novelty effects could be detected, i.e., using the average activity as dependent variable. When the four treatment groups were examined in pairs (e.g. within Anoxia, compare Novel and Home), the only significant difference was found among the Home rats; the Anoxia rats had significantly higher average activity than the Control rats ($F(1, 70) = 4.856$; $p = 0.030$; $d = 0.53$).

3.2. Novelty effect on initial open-field activity

Activity level during T1 can be considered an index for emotional reactivity to a novel environment [15]. A two-way ANOVA applied to T1 activity revealed a significant Novelty by Anoxia interaction effect ($F(1, 52) = 4.828$; $p = 0.033$; $d = 0.61$, d.f. reduced due to unequal variance; Fig. 2). Specifically, among Home rats, T1 activity was significantly higher among the Anoxia rats than among the Control rats ($F(1, 70) = 5.302$; $p = 0.024$; $d = 0.55$), whereas among Novel rats, little difference between the Anoxia and Control rats was observed. Within the Anoxia condition, Novel rats had significantly lower T1 activity than the Home rats ($F(1, 25) = 6.960$; $p = 0.014$; $d = 1.05$, d.f. reduced due to unequal variance). Among the Control rats, we did not find a significant difference between the Novel and Home rats ($p > 0.2$).

3.3. Novelty effect on subsequent change from trial 2 to trial 4

A relatively more stable phase of open-field activity began from trial 2 onwards. A two-way ANOVA applied to the subsequent changes between T2 and T4 ($T4-T2$) revealed a significant Novelty by Anoxia interaction effect ($F(1, 70) = 5.949$; $p = 0.017$; $d = 0.58$; Fig. 3). Within the Home group, activity from trial 2 through trial 4 decreased in Anoxia rats, but increased in Control rats ($F(1, 70) = 14.66$; $p < 0.001$; $d = 0.92$). In contrast, within the Novel group, no significant difference between Anoxia and Control rats was found. Among the Anoxia rats, Home rats showed a marginally significant greater decrease from T2 to T4, than the Novel rats ($F(1, 70) = 3.519$; $p = 0.076$; $d = 0.45$) while

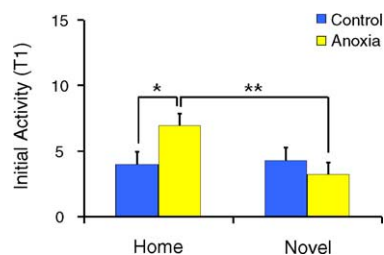


Fig. 2. Neonatal novelty exposure eliminated the effect of anoxia on the initial open-field activity (T1). Results in all figures are expressed as mean \pm S.E.M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Home Control: $n = 19$; Home Anoxia: $n = 19$; Novel Control: $n = 16$; Novel Anoxia: $n = 20$.

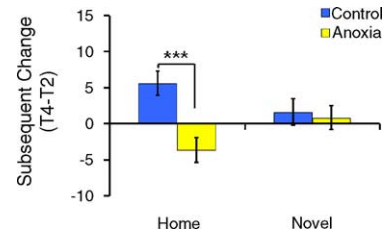


Fig. 3. Neonatal novelty exposure eliminated the effect of anoxia on subsequent changes in open-field activity ($T4-T2$).

among the Control rats, both the Novel and Home rats increased their activity but the difference did not reach statistical significance ($F(1, 70) = 2.507$; $p > 0.10$; $d = 0.38$).

3.4. Effects of anoxia on open-field measures

In addition to the Novelty related effects, the three-way ANOVA also revealed a significant main effect of Anoxia on both the distance and latency measures (distance: $F(1,70) = 4.719$; $p = 0.033$; $d = 0.52$; latency: $F(1,70) = 5.509$; $p = 0.022$; $d = 0.56$), with the Anoxia treatment increasing the distance travelled within the open field (Fig. 1a and b) and reducing latency to leave the center (Fig. 1c and d). Furthermore, the Anoxia by trial interaction effects on both the distance and latency measures were also significant (distance: $F(2.75,192) = 4.114$; $p = 0.009$; $f = 0.24$; latency: $F(2.85,199) = 4.833$; $p = 0.003$; $f = 0.26$). For the distance measure, Anoxia treatment appeared to lead to a larger increase in ambulatory activity between T1 and T2 (Fig. 1a and b); for the latency measure, Anoxia treatment appeared to produce a complementary decrease in the latency to leave the center (Fig. 1c and d).

3.5. Body weights adjusted for litter size

Previous anoxia studies reported inconsistent results concerning the effect of anoxia on body weights [12,21]. One cause of this inconsistency may be the presence of between-litter differences in body weight, which might be larger than any anoxia-induced change in body weight. Specifically, if a litter size is large, then the average body weight of that litter tends to be smaller. The two-way ANCOVA with litter size as a covariate revealed a significant Anoxia effect ($F(1, 69) = 5.186$; $p = 0.036$; $d = 0.55$) and a significant litter-size effect ($F(1, 69) = 8.524$; $p = 0.005$; $d = 0.70$). Fig. 4 shows

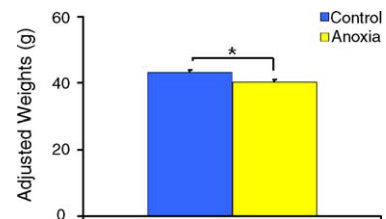


Fig. 4. Anoxia decreased body weights (adjusted for litter size using ANCOVA).

the adjusted body weight among the Anoxia rats was significantly lower than that among the Control rats, indicating that neonatal anoxia treatment resulted in lower body weight at the time of weaning. Neither the main Novelty effect nor the Anoxia by Novelty interaction effect was significant ($p > 0.2$).

4. Discussion

4.1. Neonatal novelty exposure ameliorates anoxia effect on emotional reactivity

We found that the increase in initial open-field activity, induced by neonatal anoxia on postnatal day 1, was eliminated by daily 3-min exposures to a non-home cage during postnatal day 2–21 (Fig. 2). Open-field activity has been used as an index for emotional reactivity for many decades [15]. Differences in this emotional reactivity have been used as an index for changes due to experimental manipulations, such as anoxia as well as its treatment [2,41,54,64]. Previously only among rats with moderate to severe brain damage did neonatal handling have an effect on this emotional reactivity measure [7]. Here, we replicated the anoxia-induced increase in open-field emotional reactivity. Among the Home rats, those who experienced the anoxic treatment showed significantly higher level of open-field activity. This was reflected in the significant Anoxia effects found among the Home rats both across all trials (T1–T4, Fig. 1a) and during the initial-trial (T1, Fig. 2 left). Most importantly, we showed that among rats that experienced neonatal novelty exposure, regardless of their anoxia treatment conditions, both the temporal patterns of activity across all trials and the initial activity were similar to the Control Home rats (compare Novel–Anoxia and Novel–Control against Home–Control in Fig. 1a and b; also in Fig. 2). These findings indicate that the 3-min daily experience of a change to a non-home environment was sufficient to ameliorate the effect of Anoxia on an open-field activity measure.

4.2. The role of novelty or change in environment

In the neonatal-handling procedure, handled rats differ from non-handled rats in that the handled rats are separated from the dams, experience experimenter handling, and are exposed to a relatively novel environment. In contrast, in the neonatal novelty exposure procedure, the experience of a relatively novel environment was isolated by matching the duration of maternal separation and the amount of experimenter handling between Novel and Home rats. Therefore, our design allows us to conclude that daily 3-min exposure to a change of environment alone was sufficient to trigger subsequent changes that contribute to the elimination of the anoxia effect on a measure of emotional reactivity. Regardless of the specific physical details constituting the novel environment, we believe that exposure to novelty or more generally to mild (non-traumatic) changes may be the key for trig-

gering changes that mediating the observed elimination of anoxic effects. This is consistent with the finding that transient hyperactivity, induced by a similar anoxic procedure, was prevented by exposing animals to the enriched environment manipulation [26], which typically involves changing the objects or toys regularly [20]. It is important to emphasize that instead of considering the novelty exposure as the sole cause, we favor the interpretation that neonatal novelty exposure acts only as a trigger for a complex process, which may involve a chain of events such as differential activation of the HPA axis, differential post-exposure pup–pup interactions, and differential pup–dam interactions [34].

4.3. Possible neural mechanisms underlying the elimination of anoxia effect on emotional reactivity

How neonatal novelty exposure counteracts the effect of neonatal anoxia is not known, given the behavioral nature of the present study. We speculate that the HPA axis may be a common target of the anoxia treatment and neonatal stimulation, as depicted in the theoretical diagram (Fig. 5). Open-field activity is related to circulating corticosterone [15], the end product of the HPA axis. Depletion of this hormone decreases locomotor responses in a novel open field [67], whereas its elevation above resting levels increases locomotor responses [50]. In the absence of exogenous manipulations, circulating corticosterone levels are also positively correlated with open-field activity [43,65]. Therefore, the relationship between circulating corticosterone and open-field activity can be depicted as a positively sloped line (Fig. 5 (1)). Anoxia effects can be represented by an upward shift along this line because anoxia/hypoxia leads to both elevated basal corticosterone levels ((2) [5,45]) and increased initial open-field activity ((3) [40]). In contrast, mild neonatal stimulation reduces basal corticosterone levels ((4) [23,39,42,61]) and decreases initial open-field activity ((5) [15,23]). Therefore, its effects can be represented by a downward shift along the line. These opposite directions of movement are consistent with the hypothesis that the offsetting of the anoxic effects by novelty exposure may be at least partially mediated by the reversal of the anoxic effect on HPA function. This hypothesis remains to be tested in future studies by combining the behavioral study with neuroendocrine measures.

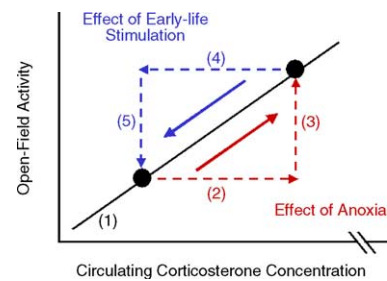


Fig. 5. A theoretical diagram depicting hypothesized opposite effects of neonatal novelty exposure and neonatal anoxia on open-field activity and circulating corticosterone concentration.

4.4. Temporal dynamics of open field behavior

We have previously shown that the temporal dynamics of open-field ambulatory activity, but not the latency measure, across multiple brief trials can be more sensitive to a subtle treatment effect [58]. This differential sensitivity of the two measures are also replicated in the present study, with the significant Novelty by Anoxia by Trial interaction effect found only on the distance measure for ambulatory activity but not on the latency measure. The reversal effect of neonatal novelty exposure on anoxia-induced changes in open-field activity was most clearly captured by the temporal patterns of activity across four trials (Fig. 1a and b).

The temporal pattern across trials may be viewed as a result of two overlapping processes: a reduction of the initial fear shortly after entering an unfamiliar environment and an increase in the familiarity of the environment. The former would be accompanied by an increase and the latter by a decrease in exploratory activity. The interaction between the two-processes can give rise to variations in the peak activity time and the rates of activity rise and fall across repeated trials. Such variations may be the reason why some researchers reported “transitory” anoxia effects on open-field activity [12,26], and why subtle differences in the interplay of the two processes can only be revealed when temporal patterns across trials are considered. This is the case for the elimination of anoxia effect by neonatal novelty exposure found in this study because neither the interaction effect between anoxia treatment nor the neonatal novelty exposure was significant when temporal patterns across trials were ignored.

This two-process conceptualization is consistent with Denenberg’s notion that in repeated open field testing, the initial and the subsequent trials are not equal as indicators for emotionality, with only the initial activity (T1) being a positive indicator of emotionality [15]. We found that among rats that experienced only their home environment, neonatal anoxia treatment increased emotional reactivity (Fig. 2). In contrast, among rats that experienced the brief change to a non-home cage, such an increase was eliminated. However, if the trial duration is sufficiently long, the initial rising phase can be masked. This could also explain why most studies only revealed the later decreasing phase of open-field activity. Therefore, we suggest that using short trial durations in combination with short inter-trial intervals allow a better chance to capture that initial rising phase, thus providing a more complete description of differences in emotional reactivity to a novel environment due to treatment conditions.

4.5. Extending effects of neonatal novelty exposure from enhancing normal function to alleviating deficit

Neonatal novelty exposure [56], as one of several possible early-life stimulation methods [14,20,33,56], has been investigated for its effects on a variety of cognitive, social,

physiological, and neurophysiological functions. When compared to their matched controls, the rats that experienced the transient exposure to a non-home cage early in life learn faster in spatial working memory tasks and have a better memory for a previously learned stimulus–reward association [56]. In social interactions, they remember previously encountered conspecifics over long durations [61]. Their basal concentration of the stress hormone corticosterone is lower [61] and their corticosterone stress response to multiple stressors is more adaptive [60]. At the cellular level, the novelty-exposed rats have greater hippocampal synaptic plasticity [63]. Hippocampal neuronal excitability and plasticity among the novelty-exposed rats are more sensitive to stress hormone modulation [70]. Finally, rats with neonatal novelty exposure also showed multiple expressions of functional brain asymmetry, reflected in “handedness” [62], turning preference [59,61], a shift in hippocampal volumetric asymmetry [68], and a selective right-sided enhancement in hippocampal LTP [63]. The present study is the first attempt in the above series of experiments to examine whether neonatal novelty exposure can also be used to counteract the effect of a clearly aversive and damaging early-life event, neonatal anoxia, which resembles birth asphyxia in humans.

4.6. Relation to other methods for alleviating anoxia effects

While several pharmacological methods, including manipulating intra-cellular calcium [41], NMDA receptors [54], adrenergic receptors [69], GABA reuptake [25], and dexamethasone [24] have been shown to counteract anoxic effects in animal models, the behavioral nature of the present method makes it a potentially viable, non-invasive, and low-cost rehabilitation method for preventing functional deficits following birth asphyxia. Hypothermia is another non-invasive treatment that can reduce anoxia/hypoxia-induced brain damage [31], typically applied shortly after anoxia/hypoxia (e.g. [52]). The brief repeated daily environmental change procedures can be viewed as subsequent extended rehabilitation therapy. Given their differences in onset time and the duration of application, these two types of methods are likely to influence the behavioral functional outcomes via different sequences of neural and physiological processes occurring at different times during development. Thus it is possible that by combining the two methods, greater benefits may be obtained. Future work is needed to determine whether and how the neonatal novelty exposure procedure can be generalized from animal models to humans.

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