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Research report

The effect of brief neonatal cryoanesthesia on physical development and adult cognitive function in mice

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HIGHLIGHTS

- We studied the effects of deep hypothermia (cryoanesthesia) on cognition in mice.
- Cryoanesthesia within 10 h after birth did not affect the body weight of pups.
- The body weight of neonatally cryoanesthetized adult mice normal.
- Up to 12 min cryoanesthesia did not affect spatial or fear memories in adult mice.
- Neonatal cryoanesthesia also did not affect the brain weight of adult mice.

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ABSTRACT

Deep hypothermia (cryoanesthesia) is often used as general anesthesia during surgery in neonatal rodents. Neonatal cryoanesthesia has been used recently to generate somatic brain transgenic (SBT) mouse models *via* intracerebral ventricular injection of rAAV vectors into both non-transgenic mice and numerous transgenic mouse models. Since, the evaluation of cognition is one of the main experimental endpoints in many of these studies, we examined the consequences of brief neonatal cryoanesthesia on the physical development and mnemonic function of adult mice. Two groups of 129FVBF1 pups from reciprocal breeding crosses underwent cryoanesthesia for 6 min (Cryo6) or 12 min (Cryo12), respectively, within the first hours (<12 h) of postnatal life. A group of pups separated from the nest and kept in ambient temperature of 33 °C for 6 min served as a control. Our results revealed that lowering the temperature of pups to ~8 °C (Cryo6) or ~5 °C (Cryo12) did not affect their body weight at pre-weaning stage and in the adulthood. The evaluation of cognitive function in adult mice revealed strong and comparable to control spatial reference, and context and tone fear memories of neonatally cryoanesthetized mice. Also, the experimental and control groups had comparable brain weight at the end of the study. Our results demonstrate that neonatal cryoanesthesia, lasting up to 12 min, has no adverse effects on the body weight of mice during development, and on their cognition in the adulthood.

1. Introduction

Deep hypothermia, also referred to as cryoanesthesia [1], is used to induce anesthesia during surgical manipulations in neonatal rodent species [1–3]. Moderate hypothermia, which significantly reduces circulatory flow, cerebrospinal fluid pressure, and perception of pain [4–6], is also used as an accessory to other anesthesia procedures [7]. In neonatal rats cryoanesthesia abolished perception of pain and significantly decreased c-fos expression in neurons [8]. Lowering body temperature to ~20 °C significantly diminished synaptic transmission in rat pups, while further drop to ~10 °C completely blocked the transmission, an effect comparable to morphine analgesia [1]. Compared to methods that use injectable anesthetic agents [2] or saturated isoflurane gas [9], neonatal cryoanesthesia can be quickly and safely induced by placing pups directly on crushed ice in paper- or aluminum foil-lined tubes or grooves (to avoid freeze skin damage), or submerging them up to their neck in ice water (directly or placing them in a laboratory glove) (see [1] for detailed discussion of these methods). Since altricial newly born mouse and rat pups cannot maintain their body temperature and are functionally poikilothermic, with thermoregulatory ability developing only during the third week of life ([10,11] cited in [1]), they are tolerant of low temperatures and can recover by re-warming, even from near 0 °C body temperature [12]. Such broad tolerance to low body temperatures of

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neonatal rodents likely represents an evolutionary adaptation to risk of bouts of hypothermia in natural environment of temperate climatic zones, when the parents leave their litters for periods of up to 4–9 h during nocturnal foraging [13].

Cryoanesthesia is often used as a common anesthetic procedure for rat pups during neonatal gonadoctomy and other hormonal manipulations [2,14], or neonatal cortical lesions [15,16]. Recently cryoanesthesia has been extensively employed during neonatal gene delivery to the brain using viral vectors [17,18] in mouse models of gene therapy or in the generation of somatic brain transgenic (SBT) mouse models of neurodegenerative diseases [19–24]. In our lab we have been generating somatic brain transgenic mouse models of Alzheimer's disease like amyloidosis [19,25] that present a new tool for elucidating mechanisms underlying amyloid β deposition in the brain [26–29]. Since many of these studies that manipulate genes expression in the brain include the evaluation of cognitive function in adult mice [30,31], we investigated here whether brief neonatal cryoanesthesia by itself can exert long lasting effects on cognition when evaluated in adulthood. Most available evidence suggests that severe hypothermia affects memory formation in mice [32,33], however, Mrosowsky reported no direct evidence for cold induced memory loss or impairments in learning reversal when hypothermia was initiated shortly after training [34]. These experiments used adult mice, longer bouts of hypothermia, and focused on relatively short-term effects of hypothermia on memory, thus were less directly related to long-term effects of neonatal cryoanesthesia on memory evaluated in adult mice. An interesting set of experiments performed in rats showed unequivocally that neonatal cryoanesthesia lasting for 60 min [35] or 9 min [36] resulted in deficits in the acquisition of the Morris water task when tested in adulthood (at 90 and 60 days postnatally, respectively). Rats and mice show relatively close developmental similarities [37], however, they are further apart with respect to behavioral similarities [38]. Therefore, in the present study we investigated whether neonatal cryoanesthesia might create a potential confounding variable affecting cognitive function in adult SBT mouse models.

Following the method adopted in our lab, which employs ~5-min long cryoanesthesia [26], we tested the effects of 6 min (Cryo6) or 12 min(Cryo12) cryoanesthesia, administered to 129FVBF1 mouse pups within ~12 h of postnatal life, on body weight during development and spatial reference and conditioned fear memories in 4–5-month old mice. Mice that underwent comparable experimental manipulation without neonatal cryoanesthesia served as a control group. Here we report that neonatal, 6- or 12-min long cryoanesthesia administered to 129FVBF1 mouse pups did not compromise their physical development or cognitive function evaluated in the adulthood.

2. Methods

2.1. Mice

FVB/NCrL (FVB) and 129S2/SvPasCrL (129) breeders were purchased from Charles River Laboratories (Wilmington, MA) at the age of 8 (females, ♀) and 9 (males, ♂) weeks. After two weeks of acclimation to the animal colony facility, reciprocal breeding trios (129♂/FVB♀♀, and FVB♂/129♀♀) were established. Apart from weekly handling, which included recording of body weight, and biweekly routine husbandry cage changes by the colony staff, the mice were not disturbed otherwise. Pregnant females, identified by substantial increase in body weight, were separated to cages supplied with nesting material (Nestlets, Code #NES3600, Ancare) and were monitored twice daily (~09:00 h and ~16:00 h) for the birth of pups. Breeders and experimental mice were housed in same-sex groups (with the exception of active breeders and pre-weanling pups) of 2–4 in ventilated mouse 29 cm × 18 cm × 13 cm cages containing 1–2 nestlets, under standard laboratory conditions (12:12 h light/dark cycle, lights on at 06:00 h, ambient room temperature of 22 °C), and water and food available *ad libitum*. The housing, husbandry and all experimental procedures were approved by the Institutional Animal Care and Use Committee of UF, and were in accordance with AAALAC and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Cryoanesthesia procedure

Newly born pups underwent cryoanesthesia procedure within 3–12 h of their postnatal life. Since the biodistribution of the AAV2 pseudotype in the brain following intracerebroventricular injection during generation of SBT models is limited if injected beyond neonatal day 1 (P1) [39], we limited the application of cryoanesthesia to the first 12 h of postnatal life. Litters were never disturbed immediately after birth, allowing the dam to lick the pups, gather them in the nest, and start nursing. Only half of a litter underwent the procedure at the time, regardless of the litter size, which minimized the disturbance and stress of the mother. The procedure took place in the adjacent to the colony room procedure suite under the ventilated hood with diffuse light. After opening the cage, the dam was encouraged to leave the nest by gentle touch on her back, if she already had not left the nest. The temperature of the nest was recorded by placing type T disk surface thermocouple probe (Cole Palmer, #K-08506-80) into a nest making sure that the surface probe was in the middle of pups huddle. Following, number of pups in the litter was counted, checked for runts, and the presence of milk in their stomach. Half of the pups were removed from the nest, and their weight, as a group, was recorded. The cage with the dam and the rest of pups was moved aside, allowing the dam continue to nurse remaining pups. Pups were cryoanesthetised on ice. An aluminum foil was placed on crushed ice and molded to form a narrow groove, wide enough to accommodate a single pup, thus maximizing its exposure to low temperature. Pups were placed in the groove in a row, preventing their body contact, the start time was recorded, and their surface body temperature was recorded every minute using the thermocouple probe. Since pups were not individually marked until the day of weaning, the body weight and the changes in surface temperature during cryoanesthesia procedure are reported as average per litter. Upon recovery from cryoanesthesia, pups were moved to a cage fitted with water thermopad lined with soft paper towels, and maintained at 33 °C, ~2 °C higher than the average nest temperature for 129 and FVB mothers recorded in our lab. During that time, pups were also warmed a few times in cupped hands, which stimulated their movement and ensured uniform recovery of each pup. Each batch of pups was returned to their home cage as a group when their movements were vigorous, the color of their body was bright pink, indicating normal blood circulation, and their surface temperature reached ~28 °C. Pups were returned to their home cages within 8–10 min after placing them in a warming cage, and in total after ~18 ± 2 min from their initial removal from the home cage. At the same time, the second half of a litter underwent cryoanesthesia. Since all breeders were routinely handled after arriving to our animal facility, the dams were familiar with experimental manipulation and readily engaged in active nursing after the pups were returned to the nest.

2.3. Experimental design

Twelve litters, born to nulliparous mothers, comprising in total of 102 pups, were allocated to the experiment. Litters were pseudo randomly allocated to 3 experimental groups: two cryoanesthesia conditions and one handling-only control condition. Since the