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The intrauterine environment affects learning ability of Tokai high avoider rat offspring derived using cryopreservation and embryo transfer-mediated reproduction

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ABSTRACT

Embryo transfer (ET) to recipient female animals is a useful technique in biological and experimental animal studies. While cryopreservation of two-cell stage rat embryos and ET to recipient rats are currently well-defined, it is unknown whether these artificial reproductive techniques and maternal factors affect offspring phenotype, particularly higher brain functions. Therefore, we assessed the effects of cryopreservation, ET, and maternal care on learning behaviour of the offspring, using Tokai high avoider (THA) rats that have a high learning ability phenotype. We found that the high learning ability of THA rat offspring was not replicated following ET to surrogate Wistar rats with a low-avoidance phenotype. Additionally, the characteristic phenotype of offspring obtained through mating of ET-derived rats was similar to that of THA rats. A postnatal cross-fostering investigation with the offspring of Wistar and THA rats showed that maternal behaviour, including postnatal care and lactation traits, did not differ between the dams of low-avoidance Wistar rats and THA rats; therefore, learning behaviour was retained in both Wistar and THA rat offspring. We conclude that the offspring phenotype, although unchanged, has an imperceptible effect on the learning ability of ET-derived THA rats through the intrauterine environment of the recipient.

1. Introduction

Embryo transfer (ET) to recipient female animals is a valuable experimental technique, particularly in studies in embryology, genetics, and biology [1]. Recent advances in genetic manipulation technologies have resulted in the generation of numerous transgenic and knockout rat strains using genome-editing techniques or embryonic stem cells [2–4]. In addition, backcrossing of genetically modified rats is conducted with rats of different genotypes [5], and new strains with multiple modified genes are also generated by intercrossing genetically manipulated strains [6]. Therefore, strain preservation of experimental rats is crucial for the reproducibility of results. Our previous studies have described the vitrification of two-cell stage rat embryos for cryopreservation and ET to recipient rats [7,8]. In surrogate conception, although embryos develop in distinct intrauterine environments, it is unknown whether maternal factors affect higher brain functions, particularly learning and memory, of offspring postpartum because prenatal learning ability of pups cannot be assessed. In addition, there are no suitable established experimental procedures to determine whether artificial reproductive technologies and maternal factors affect offspring phenotype in experimental animals.

Abbreviations: ET, embryo transfer; THA, Tokai high avoider.

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Since about 1980, we have established and maintained a unique rat strain without any genetic manipulation, Tokai high avoider (THA) rats, which were derived from JCL-Wistar rats [9–11]. THA rats were generated by selective mating of sibling rats that acquired a high avoidance rate (95%) in the lever-press Sidman avoidance task. THA rats have also shown high learning ability and few individual differences in the several behavioural tests including the Moris water maze and two-way shuttle avoidance task [9,10]. As the high learning ability of THA rats was established genetically, its effects on higher brain functions during the neural development could be appropriately evaluated. We have found that exposure to trace amounts of chemicals reduced offspring learning function during the perinatal period [12–18]. Therefore, THA rats are a useful experimental model to evaluate the effects of environmental factors on neurobehavioral development. The current study investigated the effects of assisted reproductive technologies such as embryo cryopreservation, ET, and behavioural differences of foster mothers on postnatal higher brain functions of the THA rat offspring, using learning behavioural tests. The present study may facilitate strain preservation following artificial reproductive procedures in experimental animal models.

2. Materials and methods

2.1. Animals

Male and female THA rats of the 101–105th generation, an inbred strain, were used in this study. THA rats were derived from the Jcl:Wistar rats strain and selectively bred by brother-sister mating 10-week-old rats showing a high rate of electric shock avoidance on lever-pressing tests (Fig. S1). The selection criterion for breeding was an avoidance rate of more than 95% in the lever-pressing test in the latter five sessions of a total of 10 sessions. Jcl:Wistar rats were obtained from CLEA Japan, Inc. (Tokyo, Japan). The animals were housed at an ambient temperature of 22 ± 1.0 °C, humidity 50% ± 5%, a 12:12-h light-dark cycle (lights on between 08:00 and 20:00), and *ad libitum* access to food and water (CE-2; CLEA Japan Inc. Tokyo Japan). All animals used in the present study received humane care, and all experimental protocols and procedures were reviewed, approved, and carried out in accordance with guidelines set by the Animal Experiment Committee of Tokai University and followed National Institutes of Health regulations for laboratory animal use (Permit Number 162024 and 162025).

2.2. Experimental protocol

Fig. 1 schematically represents the study design. This study comprised three distinct experiments. The first experiment was performed to detect the effect of artificial reproductive technology and surrogate delivery (experiment 1); second, to determine whether the ET-subjected dams can produce offspring and to assess the learning ability of these offspring (experiment 2); and third, to explore the effect of parental care on the THA phenotype (experiment 3).

Other materials and methods are described in supplementary data in details.

3. Results

3.1. Recipient animals of different strains affect learning ability of THA offspring

To determine whether the different strains of the recipient animal affects learning ability of the offspring, we divided ET-derived THA offspring between dams of two different strains: Wistar rats (low avoidance phenotype) and THA rats (Fig. 2A). Embryos were collected from THA female rats that were mated with male THA rats showing similarly high performance levels in the learning test (Fig. S2). The ET-derived pups were assayed for learning behaviour with lever-pressing test following the Sidman avoidance schedule at 5 weeks of age. We first compared the performance of THA pups derived from THA dams (abbreviated here as THA/THA), followed by that of THA pups derived from mating of ET-derived THA rats and observed that lesser shocks were received in each session in both male and female rats (Fig. 2B). These results indicated that embryo cryopreservation and its manipulation have limited influence on faithful reproduction of THA rats showing their characteristic high learning trait. However, the number of received shocks was significantly higher among THA pups nursed by Wistar dams with a low avoidance phenotype (abbreviated here as Wistar/THA) than among THA/THA and natural mated ET-derived THA rats groups. In addition, learning ability of Wistar/THA male pups in particular resembled that of Wistar rats even after the last session of the learning test was completed; this was an unfavourable finding (Fig. S3).

3.2. Learning ability of THA pups was restored by cross-inbreeding of embryo transfer-derived THA rats

Next, we determined whether adverse effects of ET are imprinted in the next generation. Male and female pups of ET-derived THA rats were divided into two groups on the basis of learning ability in the avoidance test: High and Low groups (Fig. S4). Their offspring were obtained by natural mating of rats with the same characteristics (High/High or Low/Low). As shown in Fig. 3, unexpectedly, there was no difference in the learning ability of pups obtained from both groups. These results suggest that the peculiar maternal environment of THA rats is one of the most important factors in retaining their high learning ability.

3.3. Distinct foster mothers do not contribute to offspring learning ability during postnatal development

In the prenatal and postnatal periods, it is assumed that maternal-foetal communication plays a pivotal role in developing higher brain functions in the offspring [19]. To determine whether different foster mothers affect learning ability of THA offspring, we performed a cross-fostering study with THA offspring being nursed by low avoidance Wistar dams and Wistar pups nursed by THA dams (Fig. 4). In contrast with learning abilities of ET-derived THA rats nursed by low avoidance Wistar dams, a postnatal cross-fostering examination did not reveal any considerable effect of different dams on learning efficiency of THA pups (foster mother Wistar/THA) compared to that of the pups of the naturally mated ET-derived THA rats. In addition, Wistar rat offspring-reared by THA dam showed no obvious major difference compared to THA rat pups (foster mother Wistar/THA). These results clearly indicated that postnatal care and lactation did not differ between Wistar and THA dams.

4. Discussion

This study revealed that the artificial reproductive technologies such as cryopreservation of two-cell stage rat embryos and ET do not affect the phenotype of THA rats. However, we found that THA pups were significantly affected by the recipient phenotype, thereby displaying a low avoidance phenotype. Thus, our results provide important and novel information on strain preservation of experimental animals and explain the importance of the intra-uterine environment in offspring development.