

Evaluation Using a Sodium Valproate-Induced Autism Spectrum Disorder (ASD) model

バルプロ酸ナトリウム誘発自閉スペクトラム症(ASD)モデルを用いた評価

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Objective

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairments in social interaction and communication, restricted interests, and repetitive stereotyped behaviors. The etiology of ASD remains elusive, but recent reports have indicated that maternal viral infection, drug intake, vitamin deficiency, and other factors during pregnancy can increase the risk of ASD in offspring.

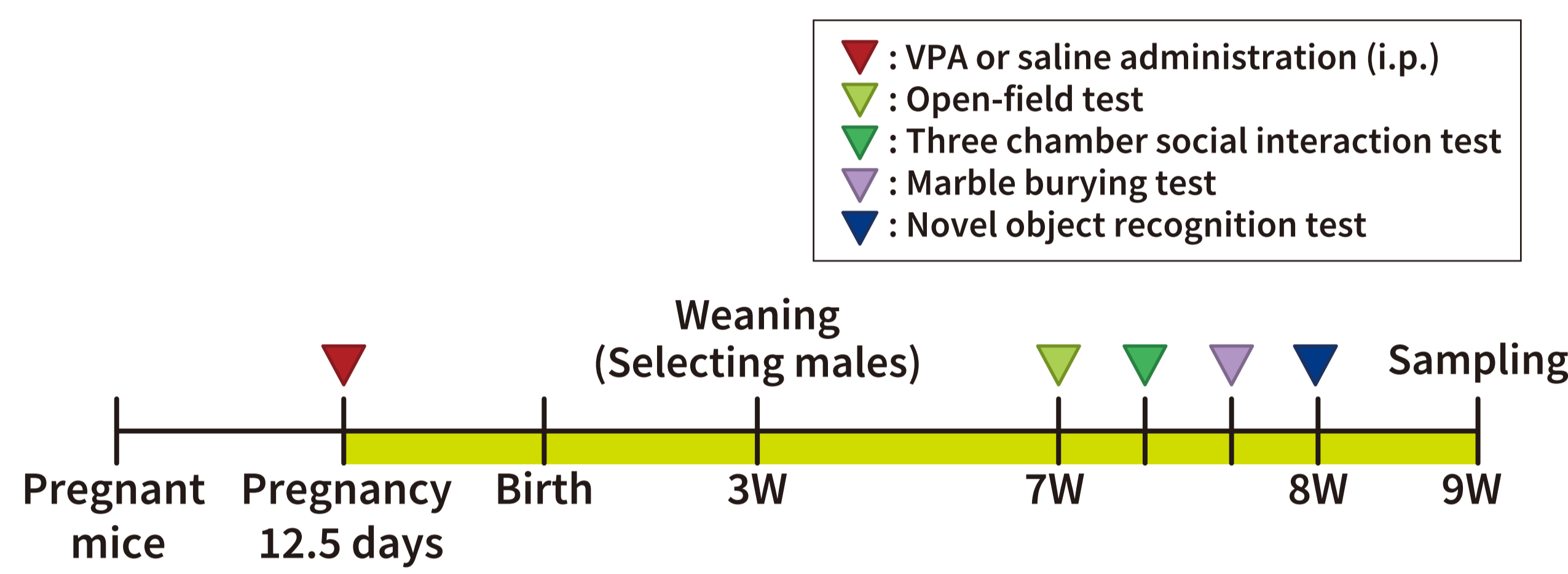
In this study, we attempted to reproduce ASD-like phenotypes using mice. Pregnant C57BL/6J mice were given a single intraperitoneal dose of valproic acid (VPA) at 500 mg/kg or 600 mg/kg on embryonic day 12.5. To examine the feasibility of this method to create an ASD model, the following behavioral characteristics of the male offspring at 7-9 weeks of age were analyzed: general activity (open field), social interaction (three-chamber test), stereotyped behavior (marble burying test), short-term memory (novel objection test) and c-Fos-positive brain regions.

Materials and Methods

- Animal**
C57BL/6J, Newborn male mice
- Model preparation**
A single dose of sodium valproate was administered on embryonic day 12.5 of pregnancy (around 1:00 p.m.).
- Test group**

Group	Strain	VPA dose (mg/kg)	Route	Timing of administration	Number of animals
Vehicle control	C57BL/6J	0	i.p.	Pregnancy 12.5 days	7
VPA 500 mg/kg		500			7
VPA 600 mg/kg		600			7

Experiment schedule



Evaluation items

- Body weight**
Measured once a week.
- Open-field test**
A cylindrical open field (80 cm in diameter and 40 cm in height) was used. Each animal was placed at the center of the open field to initiate the test. The time spent in the outside and inside zones, distance traveled in each zone, and total travel distance were measured over as 10-minute test period.
- Three-chamber social interaction test**
A three-chamber test cage (600 mm x 400 mm x 195 mm, W×D×H) was used.
 - Apparatus habituation**
Empty wire cages were placed in the left and right chambers of the three-chamber apparatus, and mice were habituated to the cage environment for 10 minute.
 - Sociability test**
A randomly selected mouse was placed in a wire cage (Animal cage), which was randomly positioned in either the left or right chamber. The wire cage in the opposite chamber was left empty (object cage). After habituation in the central chamber, the doors to the left and right chambers were opened. The behavior of the test mouse was observed and video-recorded for 10 minute, and the exploration time of each wire cage by the test mouse was quantified.
 - Social preference test**
The mouse used during the sociability test was placed in the chamber where the empty wire cage had been positioned previously (Familiar cage). A novel, randomly selected mouse was placed into the other wire cage and positioned in the opposite chamber (Stranger cage). After placing both wire cages in the left and right chambers, the doors were opened, and the test mouse was allowed to freely explore the apparatus for 10 minute. The exploration time directed toward each wire cage and each chamber was visually recorded.
- Marble burying test**
A polysulfone cage (210 mm×375 mm×196 mm, W×D×H) was used. The walls of the cage were covered with black sheets to prevent escape and to minimize external visual stimuli. Twenty glass marbles were placed on the bedding (white flakes), and the number of visible marbles was counted 10 minutes after the start of the test. The total number of buried glass marbles was taken as the MBT score.
- Novel object recognition test**
Mice were habituated to the empty apparatus (no objects placed) for 3 consecutive days (10 minutes per day). On the day following habituation, objects were placed in the apparatus, and the mice were allowed to explore freely for 10 minutes (Training trial). After a fixed interval post-training, one of the familiar objects was replaced with a novel object. The mice were then allowed to explore the apparatus freely for 5 minutes (Retention trial). The exploration time for each object and the total exploration time were measured during both the training and retention trials.
- Histopathological examination**
After exsanguination using heparinized saline under 2% isoflurane inhalation anesthesia, the harvested medial prefrontal cortex (mPFC) were immediately immersed in 4% paraformaldehyde phosphate buffer solution (4% PFA) for fixation. Subsequently, the number of c-Fos-positive cells in the fixed whole brains was detected and quantified using an anti-c-Fos antibody.

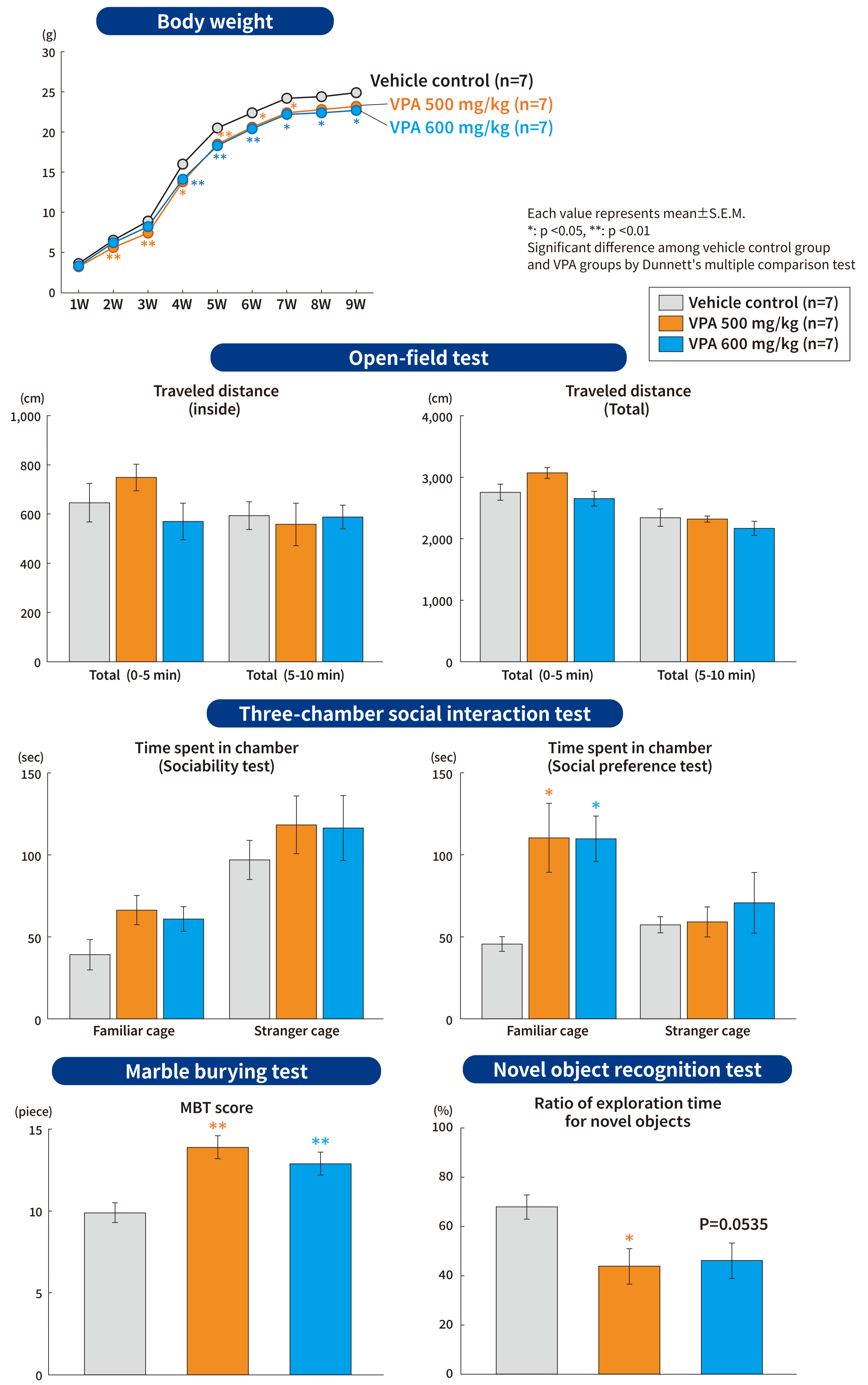
Summary in Japanese

自閉スペクトラム症 (Autism Spectrum Disorder: ASD) は社会的・コミュニケーションの障害や興味の限定、反復的な常同行動を特徴とする発達障害の1つである。ASDを発症する原因は明らかにされていないが、近年、母親の妊娠中のウイルス感染や薬物摂取、ビタミン不足などによってASDの発症リスクが増大することが報告されている。

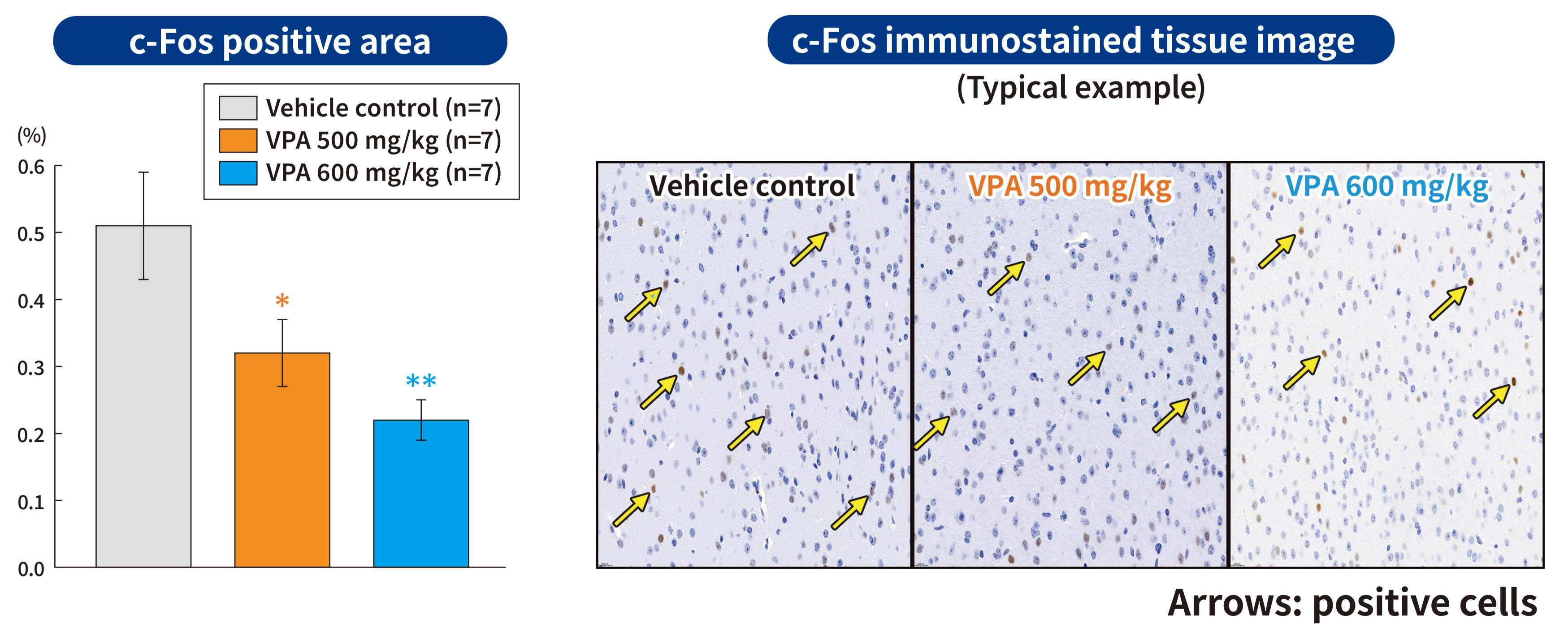
本研究では、マウスを用いてASD様症状の再現を試みた。妊娠C57BL/6Jマウスの妊娠12.5日にバルプロ酸ナトリウム (VPA) を500 mg/kgまたは600 mg/kgの用量で単回腹腔内投与し、出生した雄性マウスをASDモデルとして使用し、生後7~9週齢で評価に用いた。評価項目は、体重、オープンフィールドテストにおける活動性、3チャンバーテストにおける社会的相互作用、Marble burying testにおける常同行動とした。結果として、VPAを投与した仔マウスは体重の有意な低値、3チャンバーテストにおける社会性行動の低値、Marble burying testにおけるガラス玉埋没数の有意な高値が認められ、病理組織学的検査においては、c-Fos陽性領域の減少が認められた。これらの特徴は自閉症児で報告される症状と類似しており、本実験プロトコルはASD治療候補薬を評価する上で有用である。

Results

Functional evaluation



Histopathological examination



Conclusion

Pups born to dams treated with VPA exhibited behavioral alterations resembling those observed in children with ASD, including impaired social interaction, increased repetitive behaviors, and cognitive deficits. This experimental model appears to be a valuable tool for evaluating potential therapeutic candidates.