

# Evaluation of efficacy of anti-depressant drugs in the social defeat stress model

## 社会的敗北ストレスモデルを用いた薬効評価

○ Chika Nagao, Masana Kamioka, Yoshiyuki Hayashida, Takashi Tashiro, Kousuke Morizumi, Katsuhide Nishi, Naoyuki Hironaka

Research Unit I, Drug Discovery Innovation Center, Non-clinical Business Segment, Mediford corporation



### Objective

Social stress is thought to contribute to the development of depression in humans. Animal models exhibiting behavioral changes induced by social stress are therefore useful for evaluating antidepressant efficacy.

In this study, a social defeat stress model was established by introducing C57BL/6J mice into the territory of ICR mice and subjecting them to 10 minutes of attacks from the ICR mice for 10 days.

The effects of the social defeat stress were measured using the forced swimming test (FST) and the sucrose preference test (SPT) and social interaction score (SI score) in the social interaction test (SIT).

Furthermore, the therapeutic effects of antidepressants, imipramine (20 mg/kg), fluoxetine (20 mg/kg), and ketamine (10 mg/kg) were evaluated.

### Materials and Methods

#### Animal

Stressed mice: C57BL/6J, Male, 6 weeks old (at the start of model preparation)

Aggressor mice: Crl:CD1 (ICR), Male, 16 weeks old (at the start of model preparation)

#### Model preparation

Social defeat stress mice were prepared by introducing C57BL/6J mice into the territory of ICR mice and subjecting them to 10 minutes of attacks from the ICR mice for 10 days.

#### Group allocation

Before stress exposure, mice were allocated into a normal group and a stress model group using body weight as an index. After exposing to stress, a social interaction test (SIT) was conducted, and mice with a social interaction (SI) score of less than 100 were divided into four groups.

#### Social interaction test (SIT)

Mice were placed in an open field without an aggressor for 150 seconds, followed by an open field with an aggressor for 150 seconds. The cumulative time spent in interaction zone was recorded, and the SI score was calculated using the following formula.

SI score (%) =

$\frac{\text{[time spent in an interaction zone with an aggressor]}}{\text{[time spent in an interaction zone without an aggressor]}} \times 100$

#### Drug administration

Imipramine (20 mg/kg), fluoxetine (20 mg/kg), and ketamine (10 mg/kg) were administered intraperitoneally from day 12 to day 18. On each measurement day, the drugs were administered one hour before the measurement.

#### Forced swimming test (FST)

Mice were placed in a cylindrical tank (depth of 19 cm, temperature of  $24 \pm 1^\circ\text{C}$ ) and forced to swim for 6 minutes. The first 2 minutes were used for habituation, and the immobility period during the last 4 minutes was measured as depressive-like behavior.

#### Sucrose preference test (SPT)

Mice were simultaneously presented with a 1% sucrose solution and tap water for 2 days of training. The day after training, water and food deprivation, and then two water bottles were reinserted into the cage. The amount consumed was measured for 4 hours after the bottles were reinserted into the cage. Sucrose preference was calculated using the following formula.

The percentage of sucrose preference (%) =

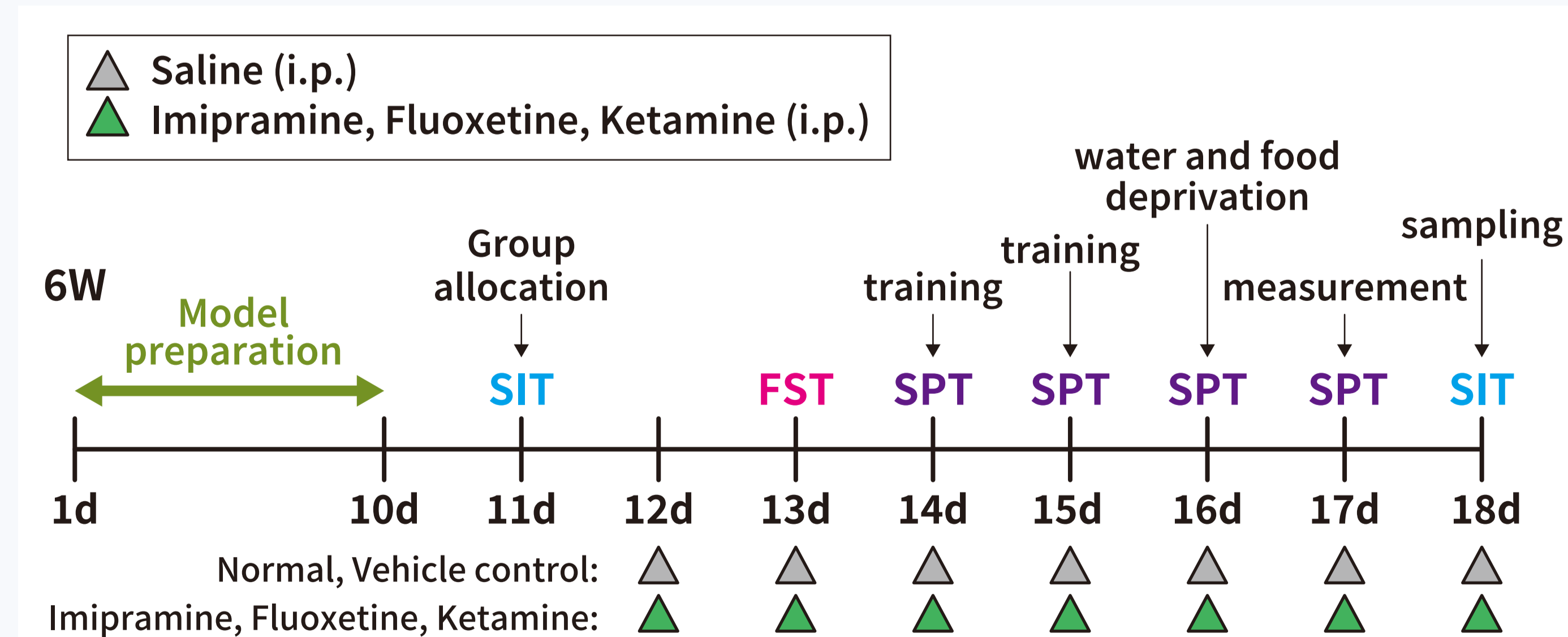
$\frac{\text{[sucrose solution consumption / total liquid consumption]} \times 100$

#### Pathological examination

After exsanguination under 2% isoflurane inhalation anesthesia, the harvested whole brains were immediately immersed in 4% paraformaldehyde phosphate buffer solution (4% PFA) for fixation. Subsequently, the number of c-Fos-positive cells in the medial prefrontal cortex was detected and quantified using an anti-c-Fos antibody. This procedure was performed on four samples from each group.

Group	Dosing article (mg/kg, i.p.)	Number of used animals	Number of adopted animals
Normal	0	16	9
Vehicle control	0	64	9
Imipramine	20		9
Fluoxetine	20		9
Ketamine	10		9

#### Experiment schedule



#### Measurement items

- Immobility time in the FST
- The percentage of sucrose preference in the SPT
- SI score of the SIT (18d)
- Histopathological examination (c-Fos-positive area)

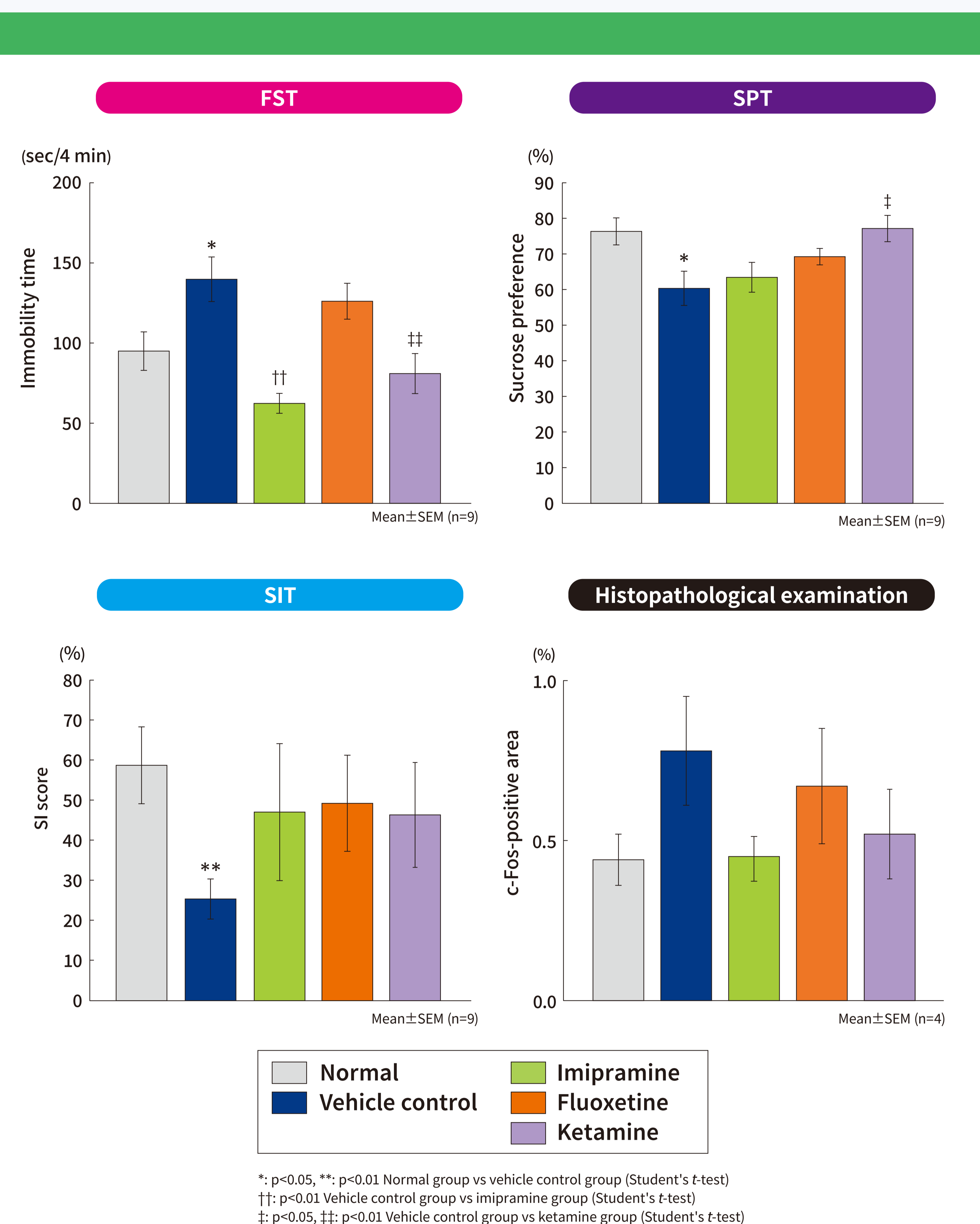
### Summary in Japanese

社会的敗北ストレスモデル (SDSモデル) とは、他個体からの継続的な身体的・精神的攻撃を受けることでヒトのうつ病と同様の身体的変化や精神症状、社会的忌避行動等を示す動物モデルである。本研究では、C57BL/6JマウスをICRマウスのテリトリーに導入し、ICRマウスから攻撃を受けさせることでSDSモデルを作製した。媒体対照群 (SDSモデル群) では、強制水泳試験における無動時間が正常群と比較して有意な高値を示した。この結果は、SDSモデル群がうつ様行動を呈することを示唆している。さらに、SDSモデル群のショ糖嗜好性および社交性テストにおける社交性スコアは、正常群と比較して有意に低下しており、うつ病の症状である報酬に対する反応低下および社会的忌避行動を呈した。

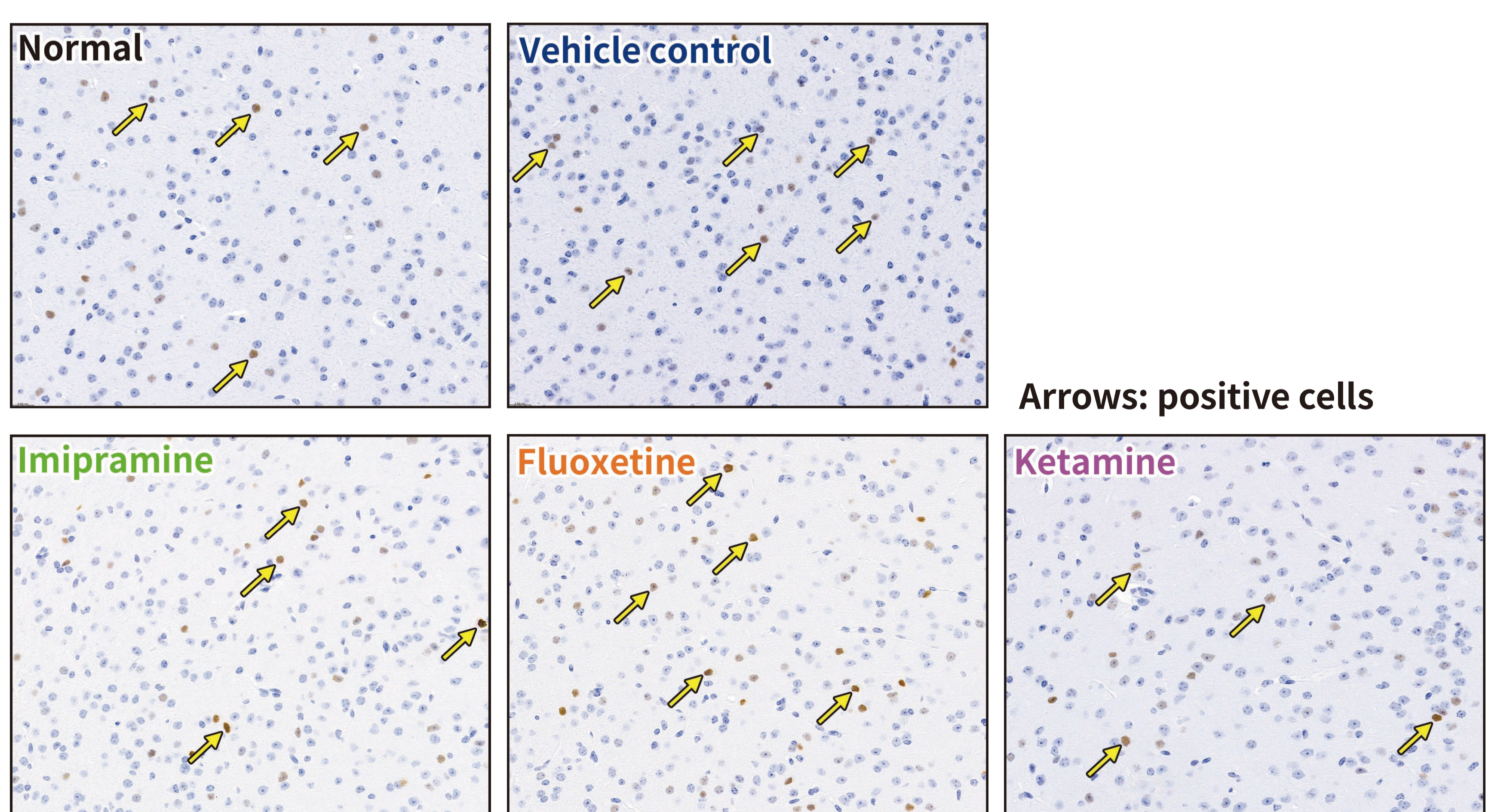
これらの特徴を示したSDSモデルにイミプラミン、フルオキセチン、ケタミンを腹腔内投与し、治療効果を評価したところ、イミプラミン群ではSDSモデル群と比較して強制水泳時の無動時間が有意に短縮した。また、ケタミン群ではSDSモデル群と比較して強制水泳時の無動時間が有意に短縮し、ショ糖嗜好性の低下も抑制された。

これらの結果より、本評価系はSDSモデルを用いた治療薬の検討に有用であると考えられる。また、各種抗うつ薬はSDSモデルが呈するうつ様行動の改善に、一定の効果を有することが示唆された。

### Results



#### c-Fos immunostained tissue image (Typical example)



### Conclusion

- By loading social defeat stress, depression-like symptoms were observed in the FST, SPT, and SIT.
- The imipramine group shortened immobility time in the FST compared with that in the vehicle control group. Furthermore, the ketamine group shortened immobility time in the FST and improved percentage of sucrose preference in the SPT compared with that in the vehicle control group.
- These findings suggest that the current social defeat stress paradigm effectively induces depression-like behaviors in mice and is suitable for assessing the efficacy of antidepressant treatments.