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SIMULTANEOUS EVALUATION METHOD FOR CENTRAL NERVOUS, CARDIOVASCULAR AND RESPIRATORY SYSTEMS IN IDENTICAL FREE-MOVING DOGS

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Introduction

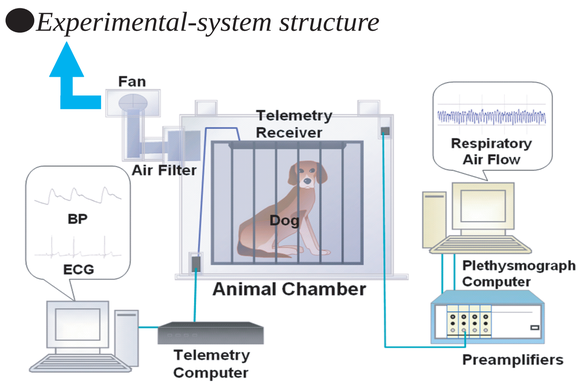
Objectives of the safety pharmacology core battery are to investigate adverse effects of new chemical entities (NCE) on vital functions, that is, the central nervous system (CNS), cardiovascular system (CV), and respiratory system (RES). Different individuals and sometimes even different animal species are generally used for each target function. For instance, Irwin or functional observational battery studies on CNS are commonly conducted using rodents; dogs and/or monkeys are most popular animals for telemetry method on CV; Whole-body-plethysmography (WBP) method using rats or blood gas analysis using dogs or monkeys is widely used for RES. It would be advantageous if effects of NCE on the said three functions are simultaneously tested in identical individuals. Specifically, it will allow us to interpret inter-functional effects of NCE in more detail and to reduce animal sacrifice, time, and cost.

We recently invented an innovative system which realizes simultaneous evaluation of NCE on CNS, CV, and RES in identical free-moving dogs. This simultaneous evaluation system has been designed to combine the observation of the general activity and behavior, telemetry method, and WBP method. We had reported that dogs could be housed in the WBP chamber, an equipment of our system, for up to 14 days, and the animals' conditions were maintained healthily in the chamber (Miyamori, et al., 2006, at the 33th Annual Meeting of the Japanese Society of Toxicology).

This study was conducted in order to investigate the feasibility of our system using a compound known to induce respiratory suppression, morphine, and two compounds known to induce QT prolongation, *dl*-sotalol and E-4031.

Methods

Experimental-system structure



Recording and measuring systems

- Video-recording system:
  HDD-DVD recorder (DMR-EH55, Panasonic Co., Ltd.), Charge-coupled device camera
- Locomotor-activity-(LMA) measuring system:
  SUPERMEX (Muromachi Kikai Co., Ltd.)
- Telemetry system:
  Open A.R.T 2.2 (Data science international), HEM ver. 3.5 (NOTOCORD Systems)
- Whole-body-plethysmography (WBP) system:
  Biosystem XA, ver.2.9.0 (Buxco Inc.)
- Blood-gas analyzer:
  ABL77 (Radiometer A/S)

Experimental items

【CNS】

- Observation of general activity and behavior
  Posture, Behavior, Convulsion, Muscle tone, Righting reflex, Pupillary reflex, Palpebral reflex, Auditory response, Touch response, Pain response, Eyes, Nose, Fur, Mucosa, Salivation, Others
- Monitoring of general activity and behavior using charge-coupled device cameras
  Posture, Behavior, Convulsion, Others
- LMA measurement using SUPERMEX
- Body-temperature measurement using telemetry system

【RES】

- WBP-data collection
  Respiration rate, Tidal volume (V_T), Minute volume (V_M), Enhanced pause (Penh)
- Measurement of blood gas
  pH, Oxygen partial pressure (PaO_2), Carbon dioxide partial pressure ($PaCO_2$), Hemoglobin oxygen saturation (SaO_2)

【CV】

- Telemetry-data collection
  Mean blood pressure (MBP), Heart rate (HR), PR interval, QRS duration, QTc interval (Fridericia's formula: QTc = QT/$RR^{1/3}$)

Animals

Three male beagle dogs were used in each experiment (Experiment 1: 19-42 months old, 11-14 kg. Experiment 2: 21 months old, 11-12 kg.). Under anesthetization with an intravenous dose of thiopental sodium (25 mg/kg), the transmitter (TL11M2-D70-PCT, Data science international) of the telemetry system was fixed in the abdominal cavity; a catheter for blood-pressure measurement was inserted into the abdominal aorta through the femoral artery; conductive wires were fixed to record AB-lead electrocardiogram; an indwelling tube for arterial-blood collection was inserted into the abdominal aorta through the femoral artery with an access port fixed on the back. In addition, as for animals used in Experiment 2, an indwelling tube for intravenous infusion was inserted in the caudal vena cava through the femoral vein with an access port fixed on the back.

Statistical analyses

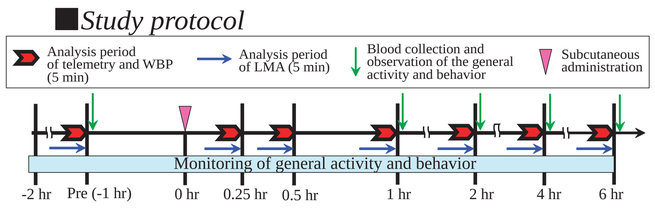
The repeated 2-way ANOVA was performed between the control group and each test-compound group in terms of measured values of the respective time points. Dunnett's test was performed, when a statistical difference between the groups was significant. The significance level was defined at 5% (2-tailed) for all the cases. As for the result of the general activity and behavior, statistical analysis was not performed.

【Experiment 1】

Administration

Morphine at the doses of 0.1, 0.3, 1, 3, and 10 mg/kg were subcutaneously administered. Saline was administered at the volume of 1 mL/kg in the same manner as a control.

Study protocol

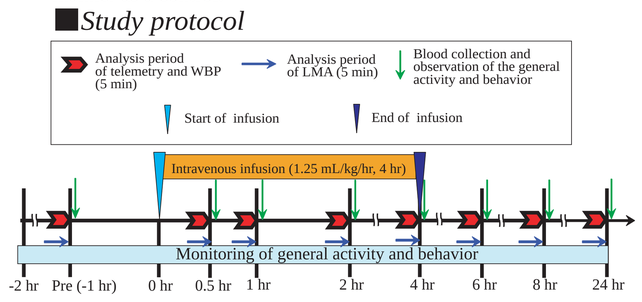


【Experiment 2】

Administration

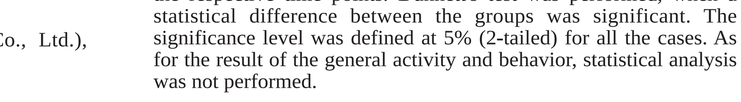
dl-Sotalol at the doses of 2.5 and 10 mg/kg/hr and E-4031 at the dose of 1 mg/kg/hr were administered continuously for 4 hours using an infusion pump (Pegasus LIGHT, PEGASUS GMBH). Saline was administered at the rate of 1.25 mL/kg/hr in the same manner as a control.

Study protocol



Results & Discussion

【Experiment 1, Morphine】



【CNS】

Table 1. Effect of morphine on general activity and behavior in dogs.

Morphine (mg/kg)	Pre (-2~0 hr)	Time after administration (hr) ~1	~2	~4	~6
Saline	NR	NR	NR	NR	NR
0.1	NR	NR	NR	NR	NR
0.3	NR	Panting (1/3)	NR	NR	NR
1	NR	Panting (3/3) Vomiting (2/3)	Panting (2/3)	NR	NR
3	NR	Panting (3/3) Vomiting (2/3) Decrease in LMA (3/3)	Panting (1/3) Decrease in LMA (3/3)	Decrease in LMA (3/3)	NR
10	NR	Panting (3/3) Vomiting (2/3) Salivation (1/3) Decrease in LMA (3/3)	Panting (1/3) Decrease in LMA (3/3)	Decrease in LMA (2/3)	Panting (1/3)

NR: Not remarkable.
Denominators represent the number of animals used in this study; numerators represent the number of the animals showed signs.

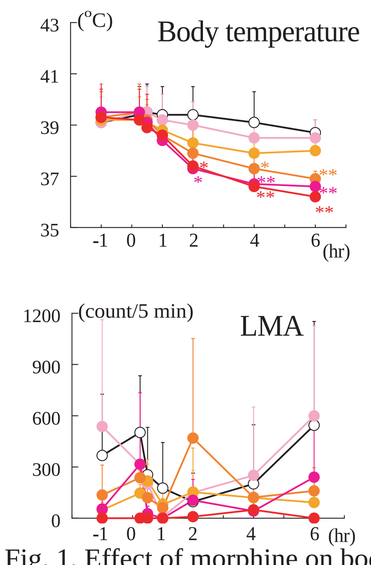


Fig. 1. Effect of morphine on body temperature and LMA in dogs.
Each value represents the mean ± S.D. of 3 dogs. *: p<0.05, **: p<0.01; Statistically significant difference vs control.

【RES】

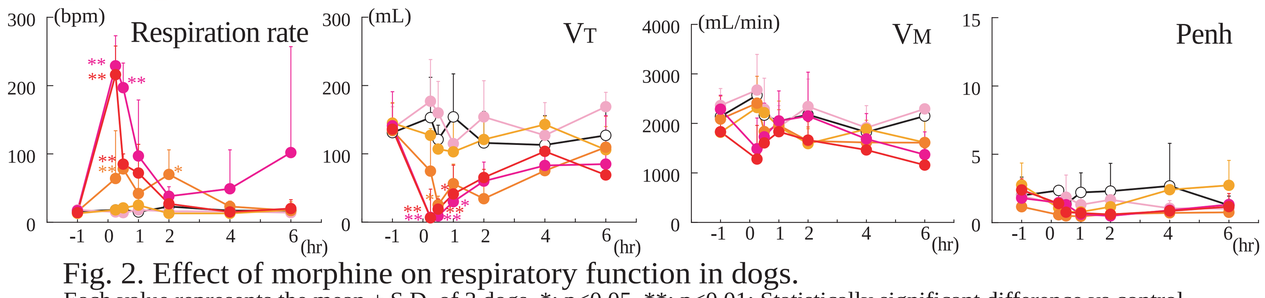


Fig. 2. Effect of morphine on respiratory function in dogs.
Each value represents the mean ± S.D. of 3 dogs. *: p<0.05, **: p<0.01; Statistically significant difference vs control.

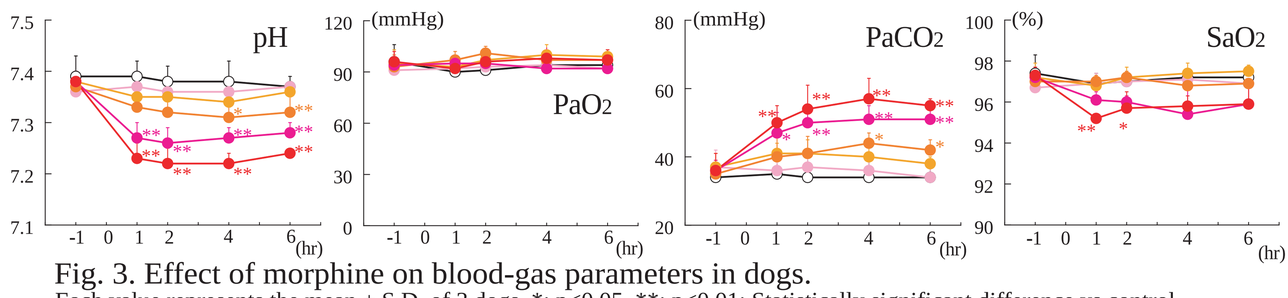


Fig. 3. Effect of morphine on blood-gas parameters in dogs.
Each value represents the mean ± S.D. of 3 dogs. *: p<0.05, **: p<0.01; Statistically significant difference vs control.

【CV】

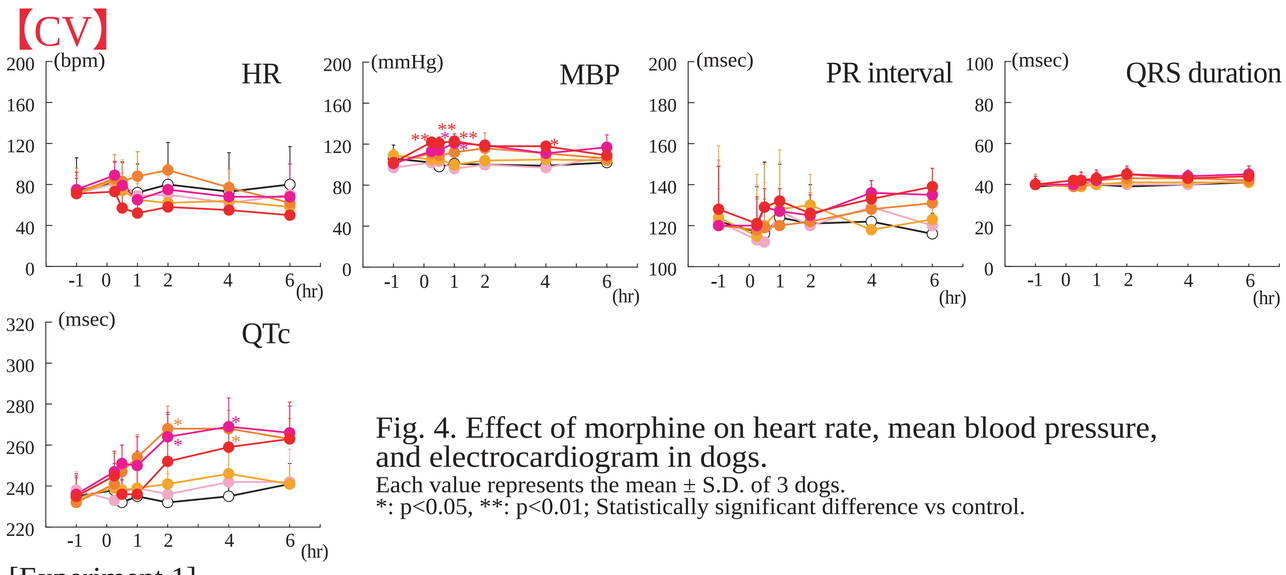


Fig. 4. Effect of morphine on heart rate, mean blood pressure, and electrocardiogram in dogs.
Each value represents the mean ± S.D. of 3 dogs.
*: p<0.05, **: p<0.01; Statistically significant difference vs control.

[Experiment 1]

Morphine at 1, 3, and 10 mg/kg showed significant increase in the respiration rate and decrease in the tidal volume when compared to the control, suggesting WBP method can monitor the effects of morphine on RES at the level compares favorably with the blood-gas analysis. In particular, the temporal resolution and sensitivity of WBP method are superior to those of the blood-gas analysis. As for the general activity and behavior, vomiting and decreased locomotor activity were observed. It was thus confirmed that the effects of morphine even on CNS was relevantly detected by our system.

【Experiment 2, *dl*-Sotalol & E-4031】

【CNS】

Table 2. Effect of 4-hour infused *dl*-sotalol and E-4031 on general activity and behavior in dogs.

Group (mg/kg/hr)	Pre (-2~0 hr)	Time after starting infusion (hr) ~0.5	~1	~2	~4	~6	~8	~24
Saline 0	NR	NR	NR	NR	NR	NR	NR	NR
dl-Sotalol 2.5	NR	Vomiting (1/3)	NR	NR	NR	NR	NR	NR
dl-Sotalol 10	NR	NR	NR	NR	NR	NR	NR	NR
E-4031 1	NR	Loose stool (1/3)	Loose stool (2/3)	NR	Loose stool (1/3)	NR	NR	NR

NR: Not remarkable.
Denominators represent the number of animals used in this study; numerators represent the number of the animals showed signs.

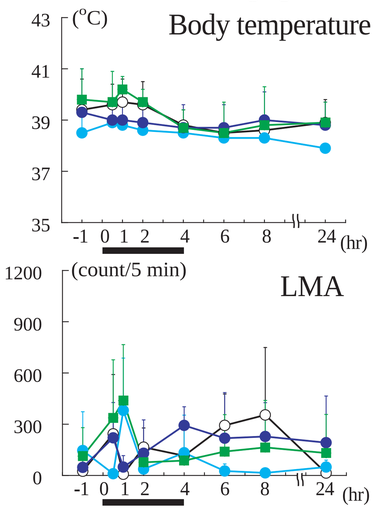


Fig. 5. Effect of 4-hour infused *dl*-sotalol and E-4031 on body temperature and LMA in dogs.
Each value represents the mean ± S.D. of 3 dogs.
Horizontal bar represents the infusion period.
There was no significant difference vs control.

【RES】

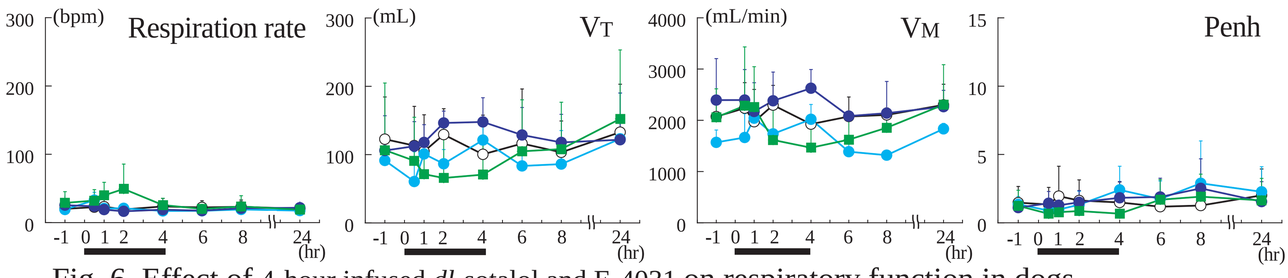


Fig. 6. Effect of 4-hour infused *dl*-sotalol and E-4031 on respiratory function in dogs.
Each value represents the mean ± S.D. of 3 dogs. Horizontal bar represents the infusion period.
There was no significant difference vs control.

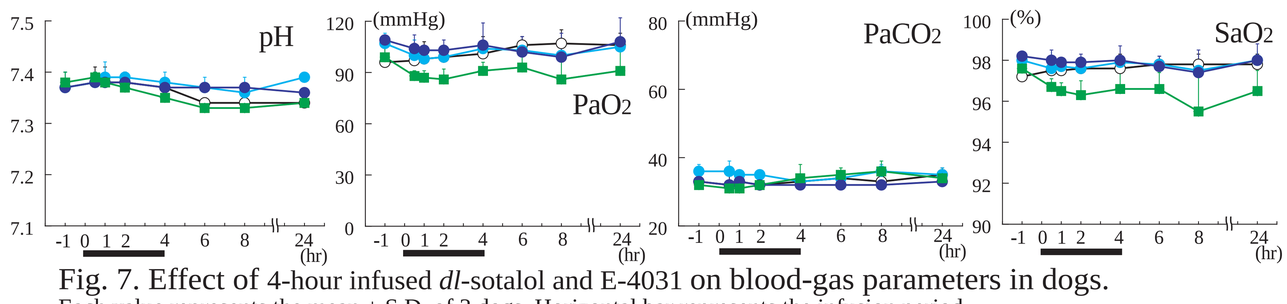


Fig. 7. Effect of 4-hour infused *dl*-sotalol and E-4031 on blood-gas parameters in dogs.
Each value represents the mean ± S.D. of 3 dogs. Horizontal bar represents the infusion period.
There was no significant difference vs control.

【CV】

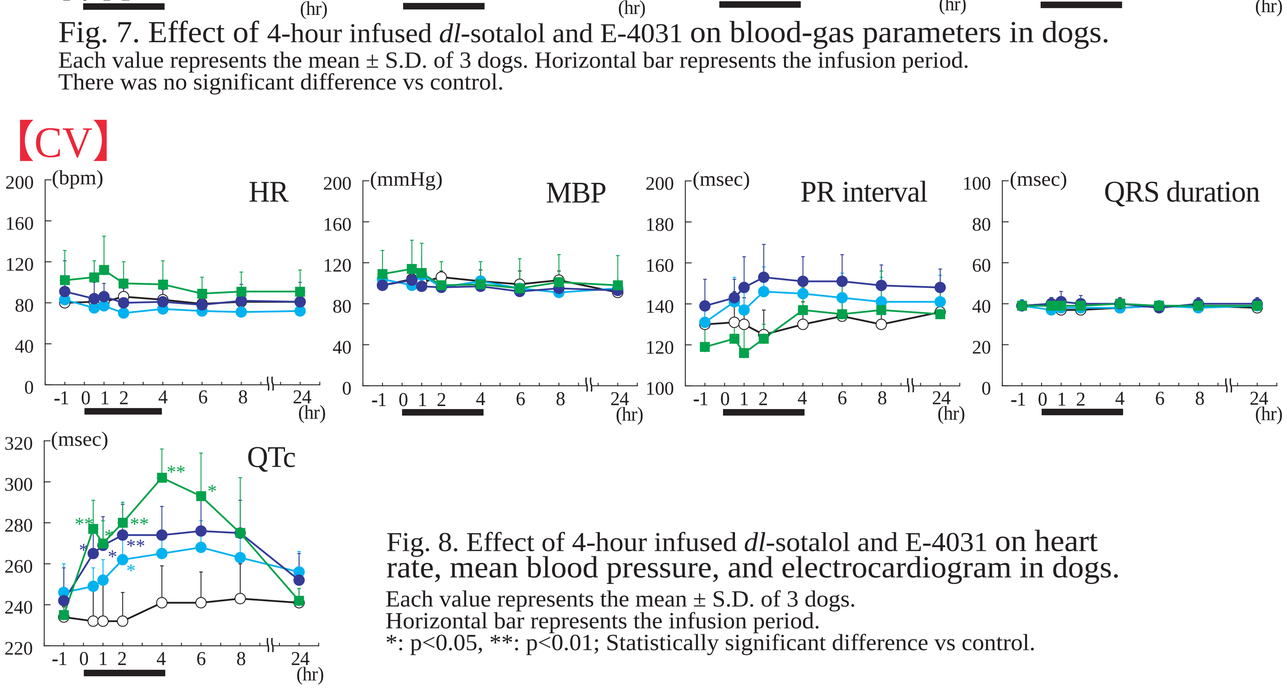


Fig. 8. Effect of 4-hour infused *dl*-sotalol and E-4031 on heart rate, mean blood pressure, and electrocardiogram in dogs.
Each value represents the mean ± S.D. of 3 dogs.
Horizontal bar represents the infusion period.
*: p<0.05, **: p<0.01; Statistically significant difference vs control.

[Experiment 2]

dl-sotalol at 10 mg/kg/hr and E-4031 at 1 mg/kg/hr showed significant prolongation of QTc interval when compared to the control. As for the effects on CNS and RES, there was no significant difference from the control. The QT-prolongation effect of *dl*-sotalol and E-4031 was properly assessed using our system.

Conclusion

The results described above suggest that our system enables to detect effects of NCE on CNS, RES, and CV simultaneously in identical free-moving dogs. In conclusion, our system could be useful for the safety pharmacology core battery.