

## Application of Supermex System to Screen Behavioral Traits Produced by Tributyltin in the Rat

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**It has been unknown whether or not endocrine disruptors exert their effects on neuronal functions, particularly leading to behavioral alterations. To address this hypothesis, we applied the Supermex system that detects the radiated body heat of an animal, representing the spontaneous motor activity of it. Intracisternal administration of tributyltin, an endocrine disruptor, into 5-day-old Wistar rats caused hyperactivity at 4–5 weeks of age. It was about 1.4 fold more hyperactive than vehicle-treated rats in the dark phase, but not in the light phase. Thus, it might be possible to apply the Supermex system to screen the behavioral deficits produced by other endocrine disruptors.**

**Key words** — Supermex, spontaneous motor activity, tributyltin

### INTRODUCTION

The fact that some endocrine disruptors affect not only the reproductive organs but also the immune system raises the possibility that they may exert their effects on neuronal functions.

There are several lines of evidence that poly-

chlorinated biphenyls (PCB) distributed in the environment cross the placenta to cause *in utero* injury to the developing brain, correlating with intelligence loss.<sup>1,2</sup> Many chemicals such as dioxins, PCB, bisphenol A, and heavy metals were detected in human umbilical cords and cord serum, suggesting that they transfer transplacentally from mother to fetus.<sup>3</sup>

Furthermore, it is still unknown that endocrine disruptors might cause behavioral trait hyperactivity seen in the related clinical disorder attention-deficit hyperactivity disorder (ADHD) and autism, whose symptoms are inattention, excess impulsivity, and uncontrolled hyperactivity.<sup>4,5</sup>

ADHD is extremely common, affecting between 3 and 7% of children.<sup>6</sup> A significant proportion of children with ADHD will continue to be affected as adolescents, and as many of 50% of these will continue to be affected as adults, making ADHD a lifelong disability. It has been hypothesized that neuroanatomical abnormality of the prefrontal cortex underlies the deficits observed in ADHD, although other brain area, particularly certain areas intimately connected to the prefrontal cortex, may be involved.<sup>7</sup>

Autism is one of a group of developmental disorders that have devastating lifelong effects on its victims.<sup>5</sup> Autism spectrum disorders affect over 400000 people in the United States.<sup>8</sup> Epidemiologic studies indicate that the number of cases of autism is increasing dramatically each year.

Whereas the etiology of both ADHD and autism is multifactorial, the possibility has been suggested that neurotoxic agents in the environment such as endocrine disruptors contribute to the incidence of these diseases.

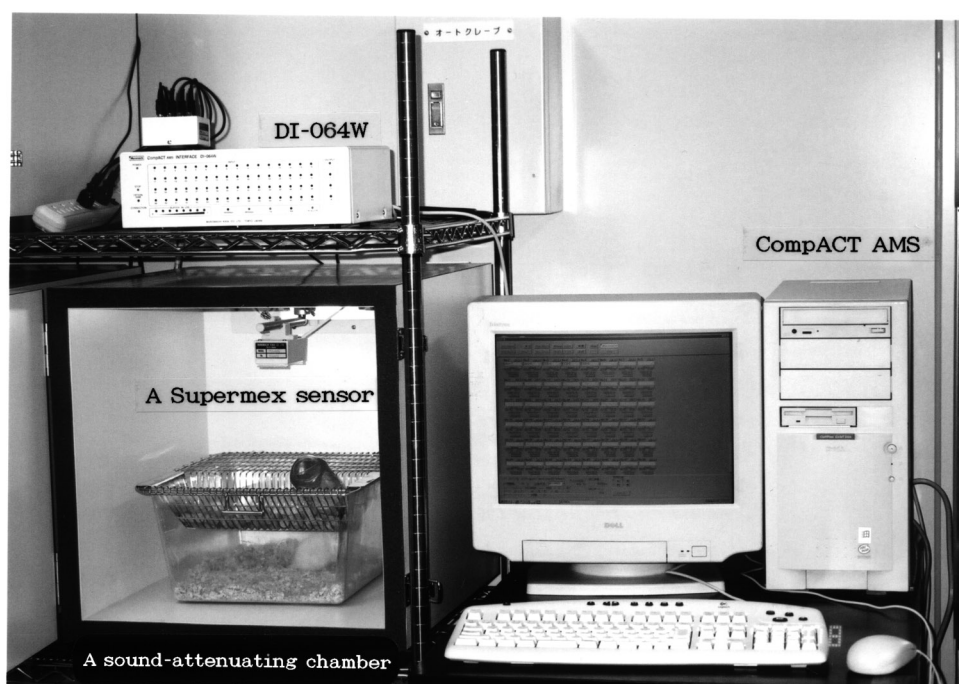
To address this hypothesis, we employed the Supermex system to examine if tributyltin, an endocrine disruptor, alter the behavioral status of the rats and found that it causes hyperactivity in the Wistar rats.

### MATERIALS AND METHODS

**Chemicals** — Tributyltin (IV) chloride was purchased from Wako Chemical Corp. (Tokyo, Japan). Olive oil was from Nakarai Chemical Corp. (Kyoto, Japan).

**Animals and Treatments with Chemicals** — Pregnant Wistar rats were obtained from Clea Japan (Tokyo, Japan). They were maintained in home cages and fed with a standard laboratory chow (MF diet,

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**Fig. 1.** The Supermex System

The general view of the system consisting of a sound-attenuating chamber, a Supermex sensor, a rat in the home cage, a 64-channel interface (DI-064W), and a personal computer with CompACT AMS software.

Oriental Yeast Corp., Tokyo, Japan) and distilled water *ad libitum* at 22°C on a light-dark cycle (12 hr/12 hr) for at least one week. Tributyltin (87 nmol) was suspended in olive oil (10  $\mu$ l), following by an intracisternal administration to a 5-day-old male rat. Control rats were injected with vehicle (10  $\mu$ l) alone. Pups were weaned at 3 weeks of age. Animals received humane care according to the NIES (Tsukuba) guidelines.

**Special equipment** — The Supermex system for behavioral measurements consist of the following equipment (Muromachi Kikai, Japan; Fig. 1).

- Supermex
- Sound-attenuating chamber (45 × 50 × 45 cm in internal width × depth × height)
- A 64-channel interface, DI-064W
- A personal computer with CompACT AMS software (Ver. 3.61)

#### **Measurements of spontaneous motor activity**

— Spontaneous motor activity of rats (4–5 weeks of age) was individually measured in a home cage by Supermex system. In this system, a sensor detects the radiated body heat of an animal. We measured the activity counted by this system during 15 min for 22–24 hr. Food and water were fully given at the beginning of counting and rats were never disturbed in any way. Ten rats were recorded concur-

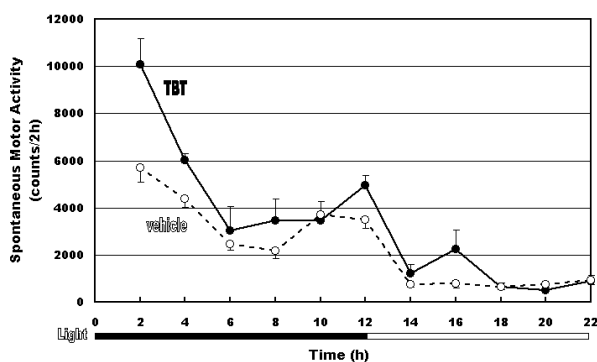
rently.

**Statistics** — Statistical analyses were carried out via Student's *t*-test using Excel Ver. 2000 software (Microsoft Corp., Tokyo, Japan).

## **RESULTS AND DISCUSSION**

Figure 1 shows the Supermex system used in this study. A Supermex sensor head consists of paired infrared pyroelectric detectors that measure the radiated body heat of the animal. This system detects any object with a temperature at least 5°C higher than background within a cone-shaped area with a 6 m diameter and a 110° vertex. The sensor monitors motion in multiple zones of the cage through an array of Fresnel lenses placed above the cage and movement of the animal in the X, Y, and Z axis can be covered.

A Supermex has the ability to analyze up to 64 channels with an optional instrument, an interface for data recording (DI-064W). Output of the sensor signals representing the magnitude of the rat's movement is transmitted by an interface device to a personal computer and is digitally converted, stored in printer memory, and processed by the CompACT AMS software.



**Fig. 2.** Behavioral Traits

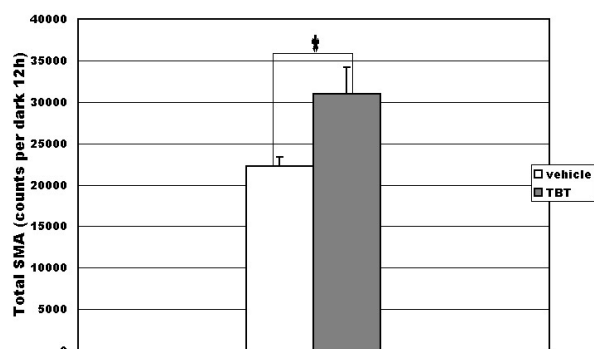
Spontaneous motor activity of 10  $\mu$ l of vehicle ( $n = 14$ ; ○)- and 87 nmol in 10  $\mu$ l of tributyltin ( $n = 5$ ; ●)-treated Wistar rats was measured by the Supermex system. Measured activity during 2 hr intervals was plotted. Data are indicated as mean  $\pm$  S.E.

Tributyltin (87 nmol) was intracisterally administered into the 5-day-old rats and their spontaneous motor activity was measured by the Supermex system at 4–5 weeks of age (Fig. 2). Control rats were given 10  $\mu$ l of olive oil alone. Tributyltin-treated rats were significantly more hyperactive than vehicle-treated rats in the nocturnal phase, but not in the diurnal phase. It was notable that the hyperactivity of the tributyltin-treated rats in the novel environment was most prominent during nighttime. The total activities of the tributyltin-treated rats for 12 hr in the dark were 1.4 times higher than those of control rats (Fig. 3) ( $p < 0.005$ ).

Weight and growth characteristics of the tributyltin-treated rats were indistinguishable from those of the solvent-treated rats (data not shown).

Thus, in this study we could detect the behavioral alterations of rats produced by an endocrine disruptor such as tributyltin using the Supermex system, a novel activity-counting system. This indicated that tributyltin certainly affected the developing brain, resulting in hyperactivity seen in the case of ADHD and autism.<sup>4,5)</sup>

Recently, Wig rats were established by gene transfer from Long-Evans Cinnamon to the Wistar king-Aptekman/Hokkaido strain. They are hyperactive and the behavioral abnormalities are transmitted by a single gene.<sup>9)</sup> Hyperactivity was shown in various knockout mice lacking gene for alpha calcium calmodulin kinase II,<sup>10)</sup> receptors of 5-hydroxytryptamine 1A, 1B,<sup>11)</sup> dopamine D4,<sup>12)</sup> or M1 muscarinic acetylcholine,<sup>13,14)</sup> dopamine transporter,<sup>15)</sup> a brain-derived neurotrophic factor,<sup>16)</sup> and Go protein.<sup>17)</sup> Endogenous factors such as thyrotropin-releasing hormone, vasoactive intestinal polypeptide,



**Fig. 3.** Total Spontaneous Motor Activities in the Nocturnal Periods

Spontaneous motor activity in nighttime shown in Fig. 2 was integrated. Data are indicated as mean  $\pm$  S.E. \*Significantly different from control rats ( $p < 0.005$ ).

and pituitary adenylate cyclase-activating polypeptide as well as 6-hydroxydopamine also cause the hyperactivity in animal models.<sup>18,19)</sup> The molecular mechanism underlying the tributyltin-induced hyperactivity remains unknown. It is tempting to examine if the neuronal transmission is modified by the chemical.

Hopefully, the Supermex system used in this study would facilitate the screening of endocrine disruptors that produce behavioral abnormalities, since its operation is very simple and sensitivity is stably higher than others.

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