Differences in Sperm Motion Between High– and Low–Shuttlebox Avoidance Rats (Hatano Strains)

MASAKO SATO, RYO OHTA, KOHICHI KOJIMA, AND MARIKO SHIROTA

From the Hatano Research Institute, Food and Drug Safety Center, Hadano, Japan.

ABSTRACT: Sperm from the caudal epididymis of 2 inbred strains of Sprague-Dawley (SD) rats, selected on the basis of their high– or low–shuttlebox avoidance responses, were analyzed for motion characteristics by a computer-assisted sperm motion analysis (CASA) system. Sperm motion in high-avoidance animals (HAA) was characterized by high velocities, high amplitude of lateral head displacement (ALH), and low beat cross frequency (BCF). Conversely, sperm from low-avoidance animals (LAA) displayed low velocities, low ALH, and high BCF. These characteristics in sperm motion were not changed by washing. Furthermore, after treatment with alpha-chlorohydrin (aCH) as a male antifertility agent affecting rat epididymal sperm motion, sperm velocities in HAA rats were signif-icantly reduced to levels similar to those in untreated LAA rats. However, ALH and BCF in HAA rats treated with aCH were different from those in untreated LAA rats. Sperm adenosine triphosphate (ATP) content was higher in HAA than in LAA rats, correlating with values of their sperm velocities. These data suggest there are apparent strain differences in sperm motion between HAA and LAA rats and that these differences are dependent on factors, including sperm energy production.

Key words: Computer-assisted sperm motion analysis, alpha-chlorohydrin, sperm velocity, adenosine triphosphate, strain differences.

J Androl 2002;23:250–258

The 2-way active avoidance learning test, known as the shuttlebox test, is commonly used in the fields of psychology, pharmacology, and behavioral teratology. This test assesses the acquisition of the ability to flee to the safe chamber after the onset of the conditioned stimulus. However, it is well known that this test produces high variability in the data from a heterogeneous animal population and, as such, makes the analysis of the effect of drugs on shuttlebox performance difficult to assess. To resolve this, some strains of rats, including the Roman strains, which have been developed as animal models, have been used (reviewed in Driscoll and Bättig, 1982). Similarly, 2 inbred strains (Hatano strains) originating from Sprague-Dawley (SD) rats (Crj:CD(SD)) have been developed at the Hatano Research Institute of Food and Drug Safety Center (Hadano, Japan) since 1985 (Ohta et al, 1995). These animals have been selectively inbred over 30 generations for rapid acquisition of a conditioned avoidance response in a shuttlebox test (high-avoidance animals [HAA]) compared with failure to acquire the response (low-avoidance animals [LAA]).

In addition to divergence in behavior, various other behavioral and phenotypic differences have also been ob-served in these animals, such as reproductive, developmental, and endocrinological characteristics. The HAA dams retrieved their pup faster and produced more milk than the LAA dams (Ohta et al, 1997). Because of these differences in dams, the body weights in the HAA infants were heavier than in the LAA infants (Ohta et al, 1998), and these body weight differences continued from weaning until mature adulthood. The adrenal weights in the HAA rats were heavier than in the LAA rats, and plasma adrenocorticotropic hormone levels after shuttlebox testing were higher in the HAA rats than in the LAA rats (Ohta et al, 1999b).

Since strain differences in female reproduction were observed, we examined sperm motion in these strains of rats at 9 weeks of age while identifying characteristics of male reproduction and unexpectedly found that there were obvious strain differences in sperm motion. In the present study, we examined whether these differences in sperm motion at 9 weeks of age were dependent on the growth differences between the HAA and LAA rats or not, and we confirmed that strain differences in sperm motion were dependent on spermatozoa. Next, sperm motion changes were examined when both strains of rats were administered alpha-chlorohydrin (aCH), an antifertility agent (Cooper and Yeung, 1999). The level of adenosine triphosphate (ATP), the energy source of sperm, was measured in intact HAA and LAA spermatozoa to investigate whether these strain differences in spermatozoa were due to their energy metabolism or not.

Materials and Methods

Animals

Male HAA and LAA rats bred in our institute were used from 9 weeks of age to 18 months of age. Animals were maintained in a light (12-hour light:12-hour dark cycle), humidity (40% to 60%), and temperature (22°C to 25°C) controlled environment, with free access to laboratory chow (CE-2; Clea Japan, Inc, Tokyo, Japan) and water.

Sperm Collection

Before experimentation, animals were anesthetized using sodium pentobarbital and killed by exsanguination. Sperm were collected as previously described by Sato et al (2000) via micropuncture of the distal cauda epididymides. The retrieved luminal contents were dispersed in prewarmed (38°C) Medium 199 with Hanks salts (Gibco, Grand Island, NY) containing 0.5% bovine serum albumin (Intergen, Purchase, NY) placed on a heating plate at 38°C, before being analyzed for sperm motion.

Sperm Motion Analysis

After approximately 5 minutes of incubation, sperm suspensions were drawn by capillary action into a cannula 100 μm deep (Microslide 1099, VitroCom Inc, Mt Lakes, NJ), and at least 200 sperm per animal, chosen from 3 to 6 random fields, were analyzed for the following parameters using the computer-assisted sperm motion analysis (CASA) system (HTM-IVOS version 10.6, Hamilton-Thorne Research, Beverly, Mass): percentage of motile sperm (%, MOT); average path velocity (μm/s, VAP); straight-line velocity (μm/s, VSL); curvilinear velocity (μm/s, VCL); amplitude of lateral head displacement (μm, ALH); and beat cross frequency (Hz, BCF). The optics were set using a 4× objective in the dark field, and the stage in the CASA system was set at 37°C. Details of other settings for the CASA system were described by Sato et al (2000).

Sperm Motion After Washing

Rats of both strains were used at 5 months of age. Sperm collection and analysis by CASA were done as described above. After the initial 5-minute incubation, a prewash analysis for sperm motion was performed using the CASA system. Then, 1-mL sperm suspensions of the remaining samples were centrifuged for 30 seconds at 1700 × *g* at room temperature. The supernatant was removed, and the sediment was suspended in 1 mL of the medium. After a 5-minute incubation following suspension of the sperm, the sperm motion was analyzed as a first washed sample. A second centrifugation and resuspension followed, and the sperm motion was analyzed for a 5-minute incubation after resuspension as a second washed sample. Therefore, the sperm motion of each rat was analyzed 3 times: after the first (prewashing), second (first washing), and third incubation (second washing). Each incubating sample was placed on a heating plate at 38°C.

aCH Administration

Rats of each strain were randomly assigned to treatment groups at 12 weeks of age and were orally administered a dose of 0 (vehicle control), 12.5, or 25 mg aCH (3-chloro-1,2-propanediol; Sigma Chemical Co, St Louis, Mo) per kg body weight. The vehicle was distilled water (Hikari Pharmaceutical Co Ltd, Tokyo, Japan), and the dosed volume was 5 mL/kg body weight based on the body weight measured on the day of administration. About 24 hours after the administration, sperm motion from each rat was analyzed as described above.

Measurement of Sperm ATP Content

The sperm were collected from caudal epididymis in HAA and LAA rats at 12 weeks of age. Minced caudal epididymis was placed into the Hanks balanced salt solution, pH 7.4, containing 10 mg/mL glucose (Gibco) at 37°C, and the sperm were dispersed. After 5 minutes of incubation, the sperm suspension was transferred to another tube, and immediately, small amounts from the sperm suspension were taken for ATP extraction and sperm motion. The remaining sperm suspensions were kept in a water bath at 37°C until the next ATP extraction and sperm motion analysis (60 minutes). Sperm motion was analyzed as described above. Sperm ATP was extracted with the 10% trichloroacetic acid (TCA) method previously described by Gottlieb et al (1987). A 50-μL sperm suspension was mixed with 50 μL TCA solution. Thereafter, 4 mL Tris-EDTA buffer (0.1 M Tris and 2 mM EDTA, pH 7.7) kept at 4°C was added and stored at −20°C until assayed. The sperm were counted with the Ident Stain kit (Hamilton-Thorne) by the CASA system (Strader et al, 1996). The ATP concentration was measured by the simple luminometric method with commercially available luciferin/luciferase reagents (ATP Detection Kit, Molecular Probes, Inc, Eugene, Ore), and bioluminescence was detected in a luminometer (Luminescence Reader BLR-201, Aloka, Japan). The ATP values were normalized to sperm counts of each sample.

Statistical Analysis

For the motion analysis studies, a mean value for each rat was calculated from each measured sperm motion, and the overall mean for each strain was determined from these individual means.

The statistical evaluation of the sperm motion parameters between 2 strains was performed by 2-way analysis of variance (strain × age). The effects of the washing and the aCH treatment on sperm motion were analyzed by 1-way analysis of variance in each strain, because we had observed apparent strain differences. When necessary, a Fisher protected least significant difference post hoc analysis (FPLSD) was used to evaluate more detail. All data are expressed as the mean plus or minus the standard deviation, and all statements of statistical significance refer to a *P*-value less than .05.

Results

Sperm Motion Analysis in HAA and LAA Rats

Visualization of the sperm on the CASA terminal screen indicated that the sperm motion characteristics of each strain were profoundly different. The sperm motion parameters are summarized in the Table, when caudal sperm

Sperm motion characteristics in HAA and LAA males analyzed by CASA*†

Age	Strain	No. of Rats Examined	Percentage of Motile Sperm	Average Path Velocity (μm/s)	Straight-line Velocity (μm/s)	Curvilinear Velocity (μm/s)	Amplitude of Lateral Head Displacement (μm)	Beat Cross Frequency (Hz)
9 weeks	HAA	4	95.5 ± 4.0	151.6 ± 8.7	107.6 ± 12.3	337.6 ± 22.8	19.4 ± 0.6	22.9 ± 0.4
	LAA	5	83.3 ± 6.5	105.3 ± 3.0	62.1 ± 3.6	231.1 ± 11.8	13.4 ± 0.4	34.1 ± 3.6
12 weeks	HAA	6	95.3 ± 3.6	160.7 ± 7.1	106.5 ± 8.7	333.2 ± 16.6	20.9 ± 0.9	21.4 ± 1.0
	LAA	7	83.1 ± 15.1	107.2 ± 7.5	63.4 ± 4.8	214.7 ± 22.2	14.3 ± 0.4	30.3 ± 3.1
15 weeks	HAA	9	92.9 ± 7.3	167.9 ± 6.8	122.9 ± 8.8	350.4 ± 12.6	20.3 ± 0.3	22.1 ± 1.0
	LAA	10	84.5 ± 5.6	110.1 ± 3.9	70.0 ± 6.2	215.5 ± 7.3	14.5 ± 0.5	28.0 ± 2.2
6 months	HAA	5	91.0 ± 4.6	151.0 ± 8.5	98.6 ± 9.5	318.9 ± 16.1	20.5 ± 0.7	20.7 ± 1.4
	LAA	5	75.8 ± 8.5	96.7 ± 5.0	59.9 ± 4.2	185.5 ± 9.8	13.1 ± 0.5	31.2 ± 2.4
12 months	HAA	6	96.7 ± 2.3	165.6 ± 4.7	117.3 ± 4.9	331.2 ± 10.0	20.3 ± 0.8	20.7 ± 0.9
	LAA	6	81.8 ± 7.7	108.3 ± 7.0	69.2 ± 5.5	200.4 ± 15.8	13.5 ± 0.8	26.6 ± 1.5
18 months	HAA	5	96.4 ± 2.6	167.1 ± 8.7	110.1 ± 2.6	339.2 ± 14.3	21.5 ± 1.2	20.1 ± 0.7
	LAA	4	70.3 ± 9.4	101.7 ± 6.6	63.7 ± 3.8	188.0 ± 14.0	13.1 ± 1.4	26.1 ± 1.3
Statistical significance (2-way analysis of variance)								
	Strain		$P < .0001$	$P < .0001$	$P < .0001$	$P < .0001$	$P < .0001$	$P < .0001$
	Age		NS	$P < .0001$	$P < .0001$	$P < .0001$	$P = .0033$	$P < .0001$
	Strain × age		NS	NS	NS	$P = .0328$	$P = .0008$	$P = .0012$

* Data are presented as the means ± SD. Sperm were obtained from the caudal epididymis and incubated in medium for 5 minutes at 38°C.
† CASA indicates computer-assisted sperm motion analysis; HAA, high-avoidance animals; LAA, low-avoidance animals; and NS, not significant.

from the HAA and LAA rats at ages from 9 weeks to 18 months were analyzed by the CASA system. The mean values of each parameter were different between the strains ($P < .0001$, Table 1), and these differences were consistently observed in rats at ages from 9 weeks to 18 months. The mean values of MOT in HAA rats were significantly higher than in LAA rats. The mean values of VAP, VSL, and VCL, which represented the swimming speed, and ALH, which represented the oscillation width of the sperm head, were much higher in the HAA rats than in the LAA rats. The mean values of BCF in the LAA rats, as shown by the oscillation rate, were significantly higher than in the HAA rats. There were also significant effects of age on all parameters, as well as significant strain–age interactions in VCL, ALH, and BCF (Table 1). However, no tendency with regard to sperm motion changes was seen that occurred on the basis of age in either strain.

The frequency distributions for each motion parameter in individual sperm collected from 5 rats at the age of 12 weeks in each strain are shown in Figure 1. In VAP, VSL, VCL, and ALH, LAA sperm constituted one population with low values, and HAA sperm constituted another with higher values. The wide-ranging distribution pattern of BCF, particularly in the LAA sperm, was different from that of other parameters. Although the mean values of sperm motion parameters were significantly different between the strains, there were similar ranges of variations in individual sperm in both strains; VAP ranged from 29.1 to 243.5 μm/s for HAA sperm and from 25.0 to 271.9 μm/s for LAA sperm; VSL ranged from 10.5 to 187.5 μm/s for HAA and from 3.8 to 171.7 μm/s for LAA; VCL ranged from 67.2 to 490 μm/s for HAA and from 42.2 to 493.2 μm/s for LAA; ALH ranged from 3.5 to 48.2 μm for HAA and from 2.6 to 39.2 μm for LAA; BCF ranged from 0 to 47.5 μm/s for HAA and from 0 to 50 μm/s for LAA.

Effects of Washing on Sperm Motion

The motion parameters in washed sperm from both strains are shown in Figure 2. The characteristics in sperm motion of both strains were observed in the sperm before washing, and these characteristics in both strains did not alter after washing. In the HAA strain, 1-way analysis of variance indicated effects of washing times on MOT, VCL, and ALH (MOT, $F_{(2,9)} = 5.80$, $P < .05$; VCL, $F_{(2,9)} = 4.50$, $P < .05$; ALH, $F_{(2,9)} = 8.86$, $P < .01$). The mean values of VCL and ALH in this strain were significantly higher (VCL, $P < .05$; ALH, $P < .01$) after washing than the values before washing. On the other hand, in the LAA strain, 1-way analysis of variance yielded values for VAP and VSL (VAP, $F_{(2,9)} = 5.67$, $P < .05$; VSL, $F_{(2,9)} = 6.51$, $P < .05$) that showed the mean values were significantly and gradually decreased by the washing. The values of MOT in both strains seemed to decrease gradually with washing times, and the mean values of MOT after the second washing were significantly lower in the HAA ($P < .01$) and the LAA ($P < .05$) than corresponding values before washing.

aCH Administration

When observing sperm motion on the CASA terminal screen for both groups given 25 mg/kg aCH, sperm swim-
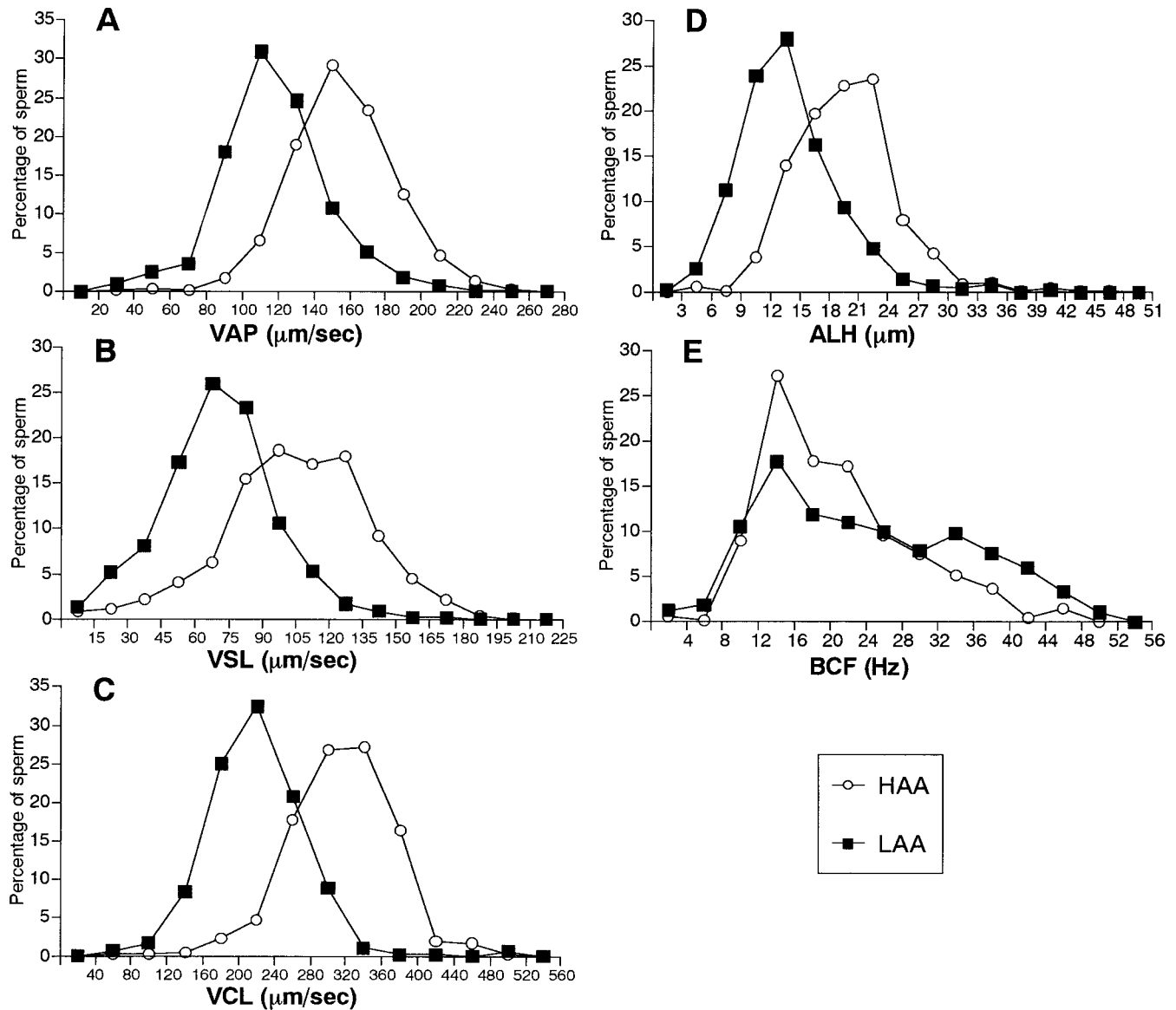
Figure 1. Comparison of frequency distribution of sperm motion parameters analyzed by computer-assisted sperm motion analysis (CASA) in individual sperm of high-avoidance animals (HAA) and low-avoidance animals (LAA) (rats) at 12 weeks of age. Sperm were collected from the population analyzed in the Table. More than 50 motile sperm (MOT) per 1 rat were randomly selected, and 466 sperm (total number examined) obtained from 5 HAA rats and 472 sperm obtained from 5 LAA rats fell within each range according to the analyzed value of individual sperm. **(A)** Average path velocity (VAP), **(B)** straight-line velocity (VSL), **(C)** curvilinear velocity (VCL), **(D)** amplitude of lateral head displacement (ALH), and **(E)** beat cross frequency (BCF).

ming speeds were clearly reduced. The sperm motion parameters in males of both strains administered aCH are shown in Figure 3. No significant difference in the mean values of MOT was observed in the 1-way analysis of variance for the aCH dose in each strain (HAA, $F(2,14) = 1.50$, $P = .26$; LAA, $F(2,14) = 0.81$, $P = .46$). In the HAA strain, 1-way analysis of variance indicated the effects of the aCH dose other parameters (VAP, $F(2,14) = 146.6$, $P < .0001$; VSL, $F(2,14) = 266.3$, $P < .0001$; VCL, $F(2,14) = 159.4$, $P < .0001$; ALH, $F(2,14) = 59.8$, $P < .0001$; BCF, $F(2,14) = 6.2$, $P = .0118$). In the HAA

strain, the mean values of VAP, VSL, and VCL in the group given 12.5 mg/kg aCH were slightly but significantly higher (VAP, $P < .05$; VSL, $P < .01$; VCL, $P < .05$) than those of the vehicle control group; however, these changes may not be due to the effects of aCH, because the values for each rat fell within the range of intact HAA rats. On the other hand, the mean values of VAP, VSL, VCL, and ALH in the HAA group given 25 mg/kg aCH were significantly lower ($P < .01$) than corresponding values of the vehicle control group. In addition, these mean values of VAP, VSL, and VCL in the HAA group
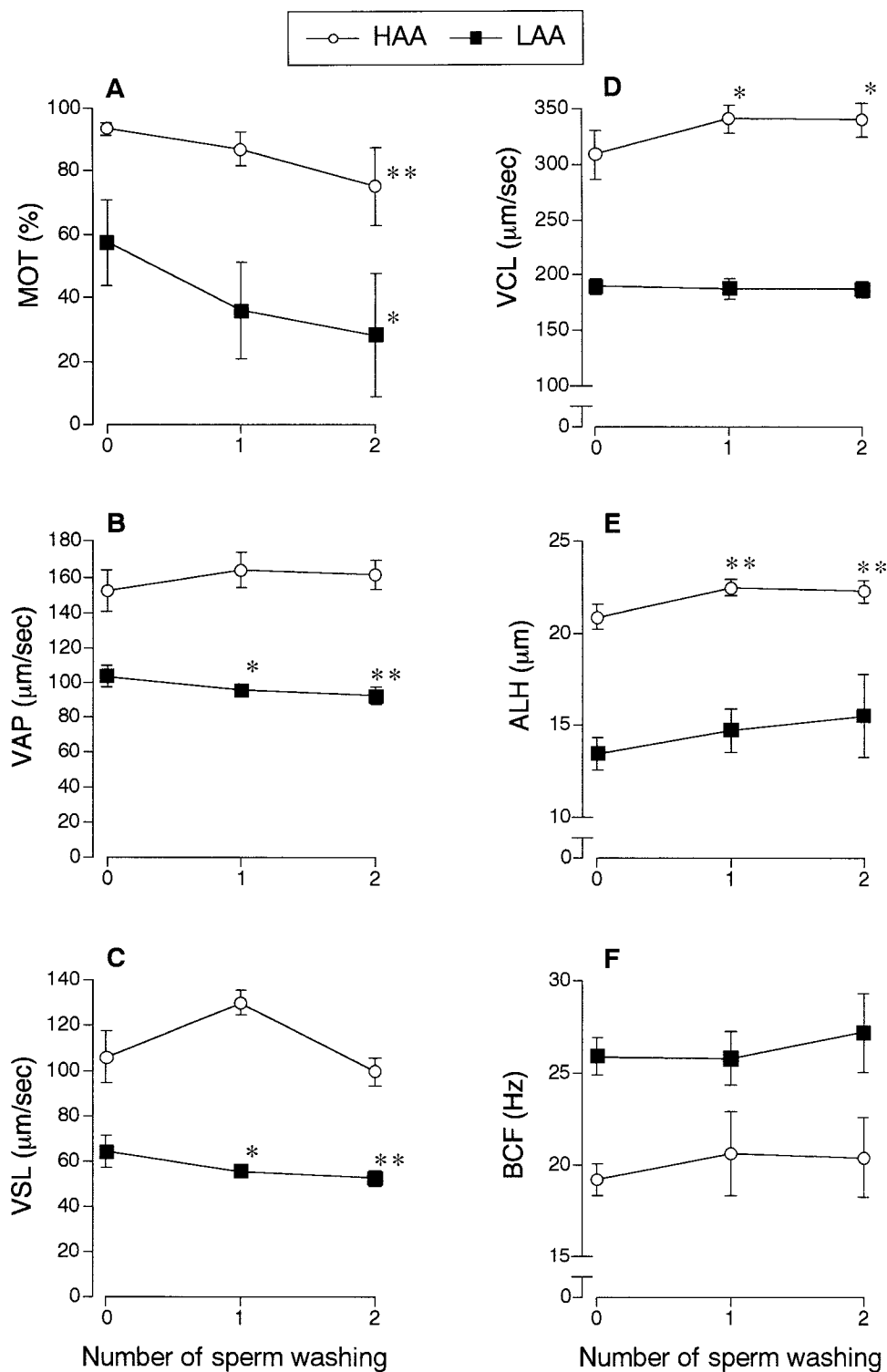
Figure 2. Changes in motion parameters in sperm of high-avoidance animals (HAA) and low-avoidance animals (LAA) (rats) analyzed by computer-assisted sperm motion analysis (CASA) before washing (0), after the first washing (1), and after the second washing (2) with a medium. Each analysis was done for 5 minutes of incubation after sampling or washing. **(A)** Percentage of motile sperm (MOT), **(B)** average path velocity (VAP), **(C)** straight-line velocity (VSL), **(D)** curvilinear velocity (VCL), **(E)** amplitude of lateral head displacement (ALH), and **(F)** beat cross frequency (BCF). Each point represents the mean plus or minus the standard deviation of 4 rats. Asterisks indicate significant differences between the values before and after washing within each strain (*, $P < .05$; **, $P < .01$).
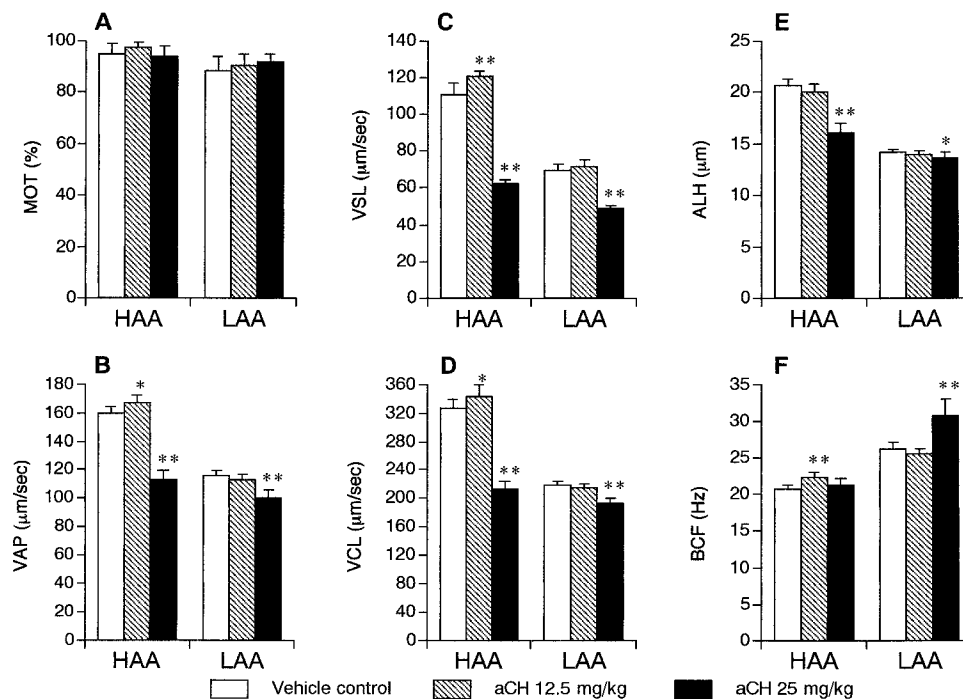
Figure 3. Sperm motion parameters analyzed by computer-assisted sperm motion analysis (CASA) in high-avoidance animals (HAA) and low-avoidance animals (LAA) (rats) 24 hours after oral administration with distilled water (vehicle control, open column), with 12.5 mg/kg alpha-chlorohydrin (aCH, hatched column), or with 25 mg/kg aCH (closed column). Sperm were obtained from the caudal epididymis and incubated in medium for 5 minutes. **(A)** Percentage of motile sperm (MOT), **(B)** average path velocity (VAP), **(C)** straight-line velocity (VSL), **(D)** curvilinear velocity (VCL), **(E)** amplitude of lateral head displacement (ALH), and **(F)** beat cross frequency (BCF). Each bar represents the mean plus or minus the standard deviation of 5 to 7 rats. Asterisks indicate significant differences from the values of the vehicle control within each strain (*, $P < .05$; **, $P < .01$).

given 25 mg/kg aCH were reduced to the levels of the corresponding values of vehicle control group of the LAA. The BCF value in the aCH 12.5 mg/kg group of HAA was significantly increased (by FPLSD, $P < .01$); however, that value in the aCH 25 mg/kg group of the same strain did not show a difference compared with the value of the vehicle control group. Also, in the LAA strain, 1-way analysis of variance indicated the effects of dose on most of the parameters, except for MOT and ALH (VAP, $F(2,14) = 17.3$, $P = .0002$; VSL, $F(2,14) = 87.5$, $P < .0001$; VCL, $F(2,14) = 27.0$, $P < .0001$; BCF, $F(2,14) = 20.9$, $P < .0001$). In the LAA rats, no significant changes in the sperm motion parameters were observed in the aCH 12.5 mg/kg group. In the aCH 25 mg/kg group of the LAA rats, the mean values of all parameters except for MOT were significantly different compared with the corresponding values of the vehicle controls by FPLSD; the mean values of VAP, VSL, VCL ($P < .01$), and ALH ($P < .05$) were significantly reduced, and the mean value of BCF was significantly increased ($P < .01$).

Sperm ATP Content and Sperm Motion

There were significant differences in both the effects of ATP content ($F(1,16) = 4.49$, $P = .042$) and incubation time ($F(1,16) = 109.4$, $P < .001$) by 2-way analysis of variance (Figure 4). The ATP content per sperm count was significantly higher in the HAA sperm than in the LAA sperm 5 minutes after collection (by FPLSD, $P < .05$). The sperm ATP contents in both HAA and LAA rats were decreased 60 minutes after collection, and there was no difference between the 2 strains. Changes in the sperm motion analyzed by CASA (Figure 5) correlated well with ATP levels in sperm with respect to time; the mean values of VAP, VSL, VCL, and ALH in both strains decreased with incubation time. Moreover, there were significant differences between the HAA and LAA sperm after 5 minutes of incubation (all parameters, $P < .01$, by FPLSD), but there were no significant differences after 60 minutes of incubation in any parameter.

Discussion

In the present study, we examined the sperm motion of HAA and LAA rats, separated on the basis of the outcome of shuttlebox avoidance rate, from 9 weeks to 18 months of age. Sperm motion differences were consistently observed between these groups during these periods. We first found that these 2 inbred strains of rats have remarkably different characteristics in epididymal sperm motion at the age of 9 weeks in both rats. There had been
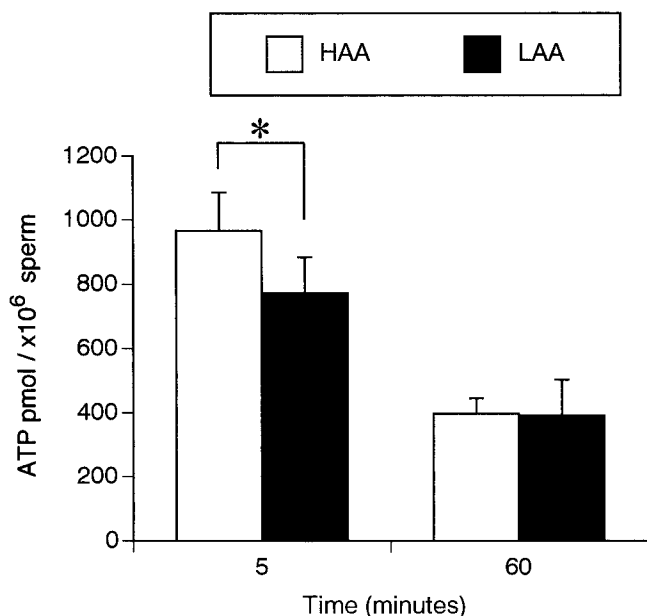
Figure 4. The adenosine triphosphate (ATP) levels in epididymal spermatozoa of high-avoidance animals (HAA; open column) and low-avoidance animals (LAA; closed column) (rats) after 5 or 60 minutes of incubation. Sperm were obtained from the caudal epididymis and incubated with Hanks balanced salt solution at 38°C. Each bar represents the mean plus or minus the standard deviation of 5 rats. Asterisks indicate significant differences between both strains (*, $P < .05$).
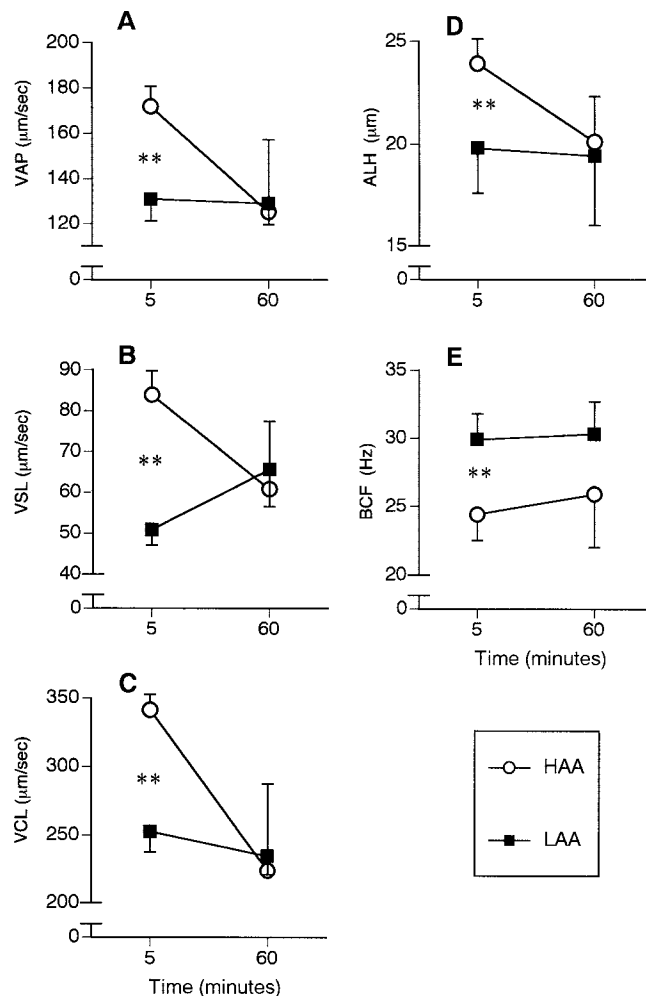

Figure 5. Sperm motion parameters analyzed by computer-assisted sperm motion analysis (CASA) in high-avoidance animals (HAA) and low-avoidance animals (LAA) (rats) after 5 or 60 minutes of incubation. Sperm were obtained from the caudal epididymis and incubated with Hanks balanced salt solution at 38°C. **(A)** Average path velocity (VAP), **(B)** straight-line velocity (VSL), **(C)** curvilinear velocity (VCL), **(D)** amplitude of lateral head displacement (ALH), and **(E)** beat cross frequency (BCF). Each bar represents the mean plus or minus the standard deviation of 5 rats. Asterisks indicate significant differences between both strains at each incubation time (**, $P < .01$).

possibilities that sperm in 9-week-old LAA rats had not yet matured, because body weights of the LAA rats were lighter than those of the HAA rats (data not shown), and the MOT in 8-week-old SD rats was less than 30% (Ohta et al, 1999a). However, the present results clearly indicate that these sperm motion differences between the HAA and LAA rats do not involve differences in their growth rate. Additionally, these strains were kept under the same conditions and in the same room; therefore, these differences in sperm motion must be dependent on genetic factor(s). The avoidance behavior of the shuttlebox test entails complicated responses; therefore, it has not been known whether these differences in sperm motion are directly linked with the genetic factors contributing to the avoidance behavior of the shuttlebox test.

The motion parameters in the individual sperm of SD rats are more similar to those of HAA sperm than to those of LAA sperm (Horimoto et al, 2000; Sato et al, 2000). The ranges of the motion parameters in individual SD rat sperm, however, were much wider than those of these 2 inbred strains, and the distributions of sperm motion in both HAA and LAA rats were included within the range of SD rat sperm motion. Sperm motion is thought to play an important role in sperm transport to the site of fertilization within the female genital tract and in sperm penetration to the oocyte investments (Yanagimachi, 1994). Both the HAA and LAA rats, however, had been maintained by natural breeding. This suggests that the sperm

motion characteristics in HAA and LAA rats had become homogeneous, and these different phenotypes of the strain diversities were within the normal range of the original SD rats.

Development of the capacity for sperm progressive motility occurs during their passage through the epididymis (Hoskins et al, 1978; Majumder et al, 1990), where sperm are subjected to a wide variety of factors (Cooper, 1998; Holland and Nixon, 1998). It was thought that sperm motion inhibitors existed in the epididymal fluid of rats, because sperm motion decreased with medium containing the epididymal fluid (Turner et al, 1978; Turner and Giles, 1982; Usselman and Cone, 1983). In this study, sperm in

the epididymal fluid were diluted and dispersed into about 400-fold volume (about 5 μL of epididymal fluid to 2 mL of medium), and even after the epididymal fluid was almost removed by washing, the sperm in both strains maintained the motion characteristics of their sperm. We suggest, therefore, that these strain differences in sperm motion characteristics are not directly induced by the factors within the luminal fluid of the caudal epididymis and that these sperm motion differences may already be established upon their arrival in the caudal epididymis.

Since aCH is thought to inhibit glyceraldehyde 3-phosphate dehydrogenase in sperm, oral administration with aCH may inhibit sperm energy production, with a resultant decrease in sperm progressive motion (Mohri et al, 1975; Ford and Rees, 1990; Jones, 1998; Cooper and Yeung, 1999; Jones and Cooper, 1999). Sperm produce ATP by a glycogenesis pathway, which is the source of their motile energy. In rats, the sperm ATP content and sperm progressive velocity were decreased in the absence of an appropriate substrate for glycolysis (Jeulin and Soufir, 1992). A report by Jelks et al (2001) showed that ATP levels in sperm from aCH-treated rats (>10 mg/kg aCH) were significantly lower when diluted in a medium containing glucose. Since we used Medium 199 containing glucose in the present study and sperm velocities (VAP, VSL, and VCL) were reduced in both strains that were administered 25 mg/kg aCH, sperm ATP levels could be reduced in both HAA and LAA rats treated with aCH. Furthermore, the sperm ATP content in the intact HAA rats was significantly greater than that of the intact LAA rats after 5 minutes of incubation. At this time, the sperm motion in the HAA and LAA rats was different, as described above; however, after 60 minutes of incubation, neither sperm ATP content nor sperm motion showed strain differences. From these results, sperm ATP content may be contributing to strain differences in sperm motion between the HAA and LAA rats.

The study by Jeulin and Soufir (1992) showed that in the presence of a substrate for glycolysis, the ATP content of rat spermatozoa decreased, but the sperm progressive velocity and beat frequency did not change. In the present study, changes in the sperm ATP content and sperm motion in the LAA rats resembled the data of SD rats (Jeulin and Soufir, 1992). However, in the HAA rats, there was good correlation between the decreased ATP levels in sperm and their decreased velocities (VAP, VSL, and VCL) and ALH. Although there were differences in some experimental conditions (eg, the gas condition for incubation), Hatano rats (the HAA or LAA rats) may have characteristics different from SD rats in the energy metabolism and sperm motion.

In addition to the strain differences in sperm swimming velocities, the HAA and LAA rats have differences in sperm swimming patterns (ALH and BCF). The sperm

treated with 25 mg/kg aCH in HAA rats had reduced velocities, with values similar to those of controls in the LAA rats. On the other hand, in the HAA sperm treated with 25 mg/kg aCH, ALH and BCF values (the values of the parameters representing the sperm motile pattern) were not similar to those of the controls in LAA rats. The BCF values for 60 minutes after incubation were clearly different between the 2 groups. The strain differences in the sperm velocities may principally depend on the sperm energy production, but the property of the energy production or metabolism affected by aCH may not be the only factors determining the sperm motile pattern in these strains.

There has been no information about the relationship between sperm motion and active avoidance responses. ATP is a source of cyclic adenosine $3',5'$-monophosphate (cAMP). It is well established that cAMP regulates not only initiation and maintenance of sperm motility (Ford and Rees, 1990; Tash, 1990) but also that cAMP is one of the second messengers for receptor-mediated, postsynaptic signal transduction (Nicoll et al, 1990). Florio et al (1999) reported that daily administration of alpha-glycerylphosphorylethanolamine to rats caused a significance improvement in their conditioned avoidance responses in an active avoidance test (shuttlebox). This compound increases the neurotransmitter activity by improving the efficiency of receptor-effector coupling through an increase in membrane fluidity. In vitro cAMP production in the brain of rats administered this compound was activated in the presence of receptor-mediated stimulation (isoproterenol or carbamyl choline). Additionally, rolipram, which is a cAMP-specific phosphodiesterase 4 inhibitor, increased brain cAMP levels (Schneider, 1984) and ameliorated scopolamine-induced impairments of learning and memory in rats (Imanishi et al, 1997). Although the above studies did not address sperm motion, the functional differences that exist between HAA and LAA with respect to sperm motion and active avoidance responses may also be explained by differences in cAMP levels.

In conclusion, these Hatano strains, the HAA and LAA rats, have obvious differences in motion characteristics of the cauda epididymal spermatozoa genetically, and the sperm motion differences may be dependent on their sperm ATP content. Therefore, these 2 inbred strains of rats may become useful animal models, not only for behavioral teratology but also for the study of physiology or toxicology on sperm motion. Moreover, the clarification of these strain differences in sperm motility may add to our knowledge of the mechanism of sperm motion.

References

Cooper TG. Interactions between epididymal secretions and spermatozoa. *J Reprod Fertil.* 1998;53(suppl):119–136.

Cooper TG, Yeung CH. Recent biochemical approaches to post-testicular, epididymal contraception. *Hum Reprod Update.* 1999;5:141–152.

Driscoll P, Bättig K. Behavioral, emotional and neurobehavioral profiles of rats selected for extreme differences in active, two-way avoidance performance. In: Lieblich I, ed. *Genetics of the Brain.* Amsterdam: Elsevier Biomedical Press; 1982:95–123.

Florio T, Bajetto A, Thellung S, Arena S, Corsaro A, Bonavia R, Merlino M, Schettini G. Prolonged treatment with alpha-glycerylphosphorylethanolamine facilitates the acquisition of an active avoidance behavior and selectively increases neuronal signal transudation in rats. *Aging (Milano).* 1999;11:335–342.

Ford WCL, Rees JM. The bioenergetics of mammalian sperm motility. In: Gagnon C, ed. *Controls of Sperm Motility: Biological and Clinical Aspects.* Boca Raton, Fla: CRC Press; 1990:175–202.

Gottlieb C, Svanborg K, Eneroth P, Bygdeman M. Adenosine triphosphate in human semen: a study on conditions for a bioluminescence assay. *Fertil Steril.* 1987;47:992–999.

Holland MK, Nixon B. The specificity of epididymal secretory proteins. *J Reprod Fertil.* 1998;53(suppl):197–210.

Horimoto M, Isobe Y, Isogai Y, Tachibana M. Rat epididymal sperm motion changes induced by ethylene glycol monoethyl ether, sulfasalazine, and 2,5-hexanedione. *Reprod Toxicol.* 2000;14:55–63.

Hoskins DD, Brant H, Acott TS. Initiation of sperm motility in the mammalian epididymis. *Fed Proc.* 1978;37:2534–2542.

Imanishi T, Sawa A, Ichimaru Y, Miyashiro M, Kato S, Yamamoto T, Ueki S. Ameliorating effects of rolipram on experimentally induced impairments of learning and memory in rodents. *Eur J Pharmacol.* 1997;321:273–278.

Jelks K, Berger T, Horner C, Miller MG. Alpha-chlorohydrin induced changes in sperm fertilizing ability in the rat: association with diminished sperm ATP levels and motility. *Reprod Toxicol.* 2001;15:11–20.

Jeulin C, Soufir, JC. Reversible intracellular ATP changes in intact rat spermatozoa and effects on flagellar sperm movement. *Cell Motil Cytoskeleton.* 1992;21:210–222.

Jones AR. Chemical interference with sperm metabolic pathways. *J Reprod Fertil.* 1998;53(suppl):227–234.

Jones AR, Cooper TG. A re-appraisal of the post-testicular action and toxicity of chlorinated antifertility compounds. *Int J Androl.* 1999;22:130–138.

Majumder GC, Dey CS, Haldar S, Barua M. Biochemical parameters of initiation and regulation of sperm motility. *Arch Androl.* 1990;24:287–303.

Mohri H, Suter DA, Brown-Woodman PD, White IG, Ridley DD. Identification of the biological lesion by alpha-chlorohydrin in spermatozoa. *Nature.* 1975;255:75–77.

Nicoll RA, Malenka RC, Kauer JA. Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system. *Physiol Rev.* 1990;70:513–565.

Ohta R, Matsumoto A, Hashimoto Y, Nagao T, Mizutani M. Behavioral characteristics of rats selectively bred for high and low avoidance shuttlebox response. *Congenital Anom.* 1995;35:223–229.

Ohta R, Matsumoto A, Nagao T, Mizutani M. Comparative study of behavioral development between high and low shuttlebox avoidance rats. *Physiol Behav.* 1998;63:545–551.

Ohta R, Matsumoto A, Shirota M, Nagao T, Tohei A, Taya K. Maternal behavior in high and low shuttlebox avoidance lines of rats. In: *Proceedings of the 13th International Congress of Comparative Endocrinology.* Bologna: Monduzzi Editore SpA; 1997:1771–1775.

Ohta R, Sato M, Saitoh Y, Nagata T, Horiuchi S, Kojima K. Comparison of testicular histopathology and sperm evaluation between Crj:CD(SD) and Crj:CD(SD)IGS rats. In: Matsuzawa T, Inoue H, eds. *Biological Reference Data on Crj:CD(SD)IGS Rats—1999.* Tokyo: Best Printing; 1999a:217–220.

Ohta R, Shirota M, Adachi T, Tohei A, Taya K. Plasma ACTH levels during early, two-way avoidance acquisition in high- and low-avoidance rats (Hatano strains). *Behav Genet.* 1999b;29:137–144.

Sato M, Ohta R, Wada K, Marumo H, Shirota M, Nagao N. Utilization of a computer-assisted sperm motion analysis system to examine effects of dinoseb on rat sperm. *J Reprod Dev.* 2000;46:279–286.

Schneider HH. Brain cAMP response to phosphodiesterase inhibitors in rats killed by microwave irradiation or decapitation. *Biochem Pharmacol.* 1984;33:1690–1693.

Strader LF, Linder RE, Perreault SD. Comparison of rat epididymal sperm counts by IVOS HTM-IDENT and hemacytometer. *Reprod Toxicol.* 1996;10:529–533.

Tash JS. Role of cAMP, calcium, and protein phosphorylation in sperm motility. In: Gagnon C, ed. *Controls of Sperm Motility: Biological and Clinical Aspects.* Boca Raton, Fla: CRC Press; 1990:229–240.

Turner TT, D'Addario D, Howards SS. Further observations on the initiation of sperm motility. *Biol Reprod.* 1978;19:1095–1101.

Turner TT, Giles RD. Sperm motility-inhibiting factor in rat epididymis. *Am J Physiol.* 1982;242:R199–R203.

Usselman MC, Cone RA. Rat sperm are mechanically immobilized in the caudal epididymis by "immobilin," a high molecular weight glycoprotein. *Biol Reprod.* 1983;29:1241–1253.

Yanagimachi R. Mammalian fertilization. In: Knobil E, Neill JD, eds. *The Physiology of Reproduction.* 2nd ed. New York, NY: Raven Press; 1994:189–317.