

enzymes that may cleave the peptide at the positions of N- and C-terminal end.

#### POSTNATAL DEVELOPMENT OF SEPTO-MESENCEPHALIC CONNECTION IN RATS: TRACT-TRACING ANALYSIS WITH DI1

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In the rat, neural connection of the lateral septum (LS) and the mesencephalic central gray (MCG) is known to play an important role in regulating lordosis behavior. The number of LS neurons projecting MCG is larger in females than that in males. In the present study, to investigate the development of LS-MCG connection, a lipophilic fluorescent dye, DiI, was pasted on the cut surface of the LS of 1, 5, and 15 day-aged rats. In the birthday, a few labeled axons were found in the rostral part of the MCG. The hippocampus (HPC) also contains small amount of labeled axons. In the rat of 5 and 15 day-age, the number of labeled fibers in the MCG were still small, although increase of labeled fibers in HPC was seen according to the age. Thus, in this experiment, remarkable development of LS-MCG connection was not seen from day 1 to day 15 after birth.

#### MALE MICE HAVE BETTER MEMORY IN OBJECT RECOGNITION TEST AND ESTROGEN IMPROVES MEMORY SCORES ONLY IN FEMALES.

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By using an object recognition test (ORT), which was designed for assessment of non-spatial memory, we investigated in mice whether there is any sex difference in recognition and memory. Male and female mice were gonadectomized at 6-10 weeks of age, and received subcutaneous injections of estradiol-17 $\beta$  (E2, 1 $\mu$ g / 30 g b.w.) for 2 days beginning on 15 days after gonadectomy, then subjected to ORT on the 3<sup>rd</sup> day. Mice received vehicle injections were used as controls. Objects were composed from plastic toy blocks almost in similar height and width. We could not detect any difference in discrimination of two given objects between sexes and with or without E2 administration. However, in memory task test, male mice showed significantly higher scores than females. E2 improved the scores only in females up to those in males but not in males. It is well established that brain sex difference is due to the exposure to androgen in critical period and affects various reproductive parameters. The present observation extended the similar concept to the ability of object recognition memory and the sensitivity to estrogen treatment at adult.

#### VITELLOGENIN RELATED PROTEINS IN THE LIVER AND TESTIS OF 17 $\beta$ -ESTRADIOL EXPOSED MALE MEDAKA (*ORYZIAS LATIPES*)

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Vitellogenin (VTG) is an egg yolk precursor. It is known that VTG is produced in male medaka when they were exposed to estrogen or estrogen like endocrine disrupters. In the present study, we examined the localization of VTG in the liver and testis of 17 $\beta$ -estradiol (E2) treated male medaka by immunohistochemistry. In the liver, the cytoplasm of the cells surrounding the capillaries was immunopositive to the antiserum against medaka VTG. The intensity of immunoreactivities was stronger at the cell membrane close to the capillaries than in the cytoplasm. In the testis, the endothelial cells, probably the blood vessels surrounding the seminiferous tubules and the inside of the cells in the seminiferous tubules were immunopositive. The testis of E2 treated medaka was shown to take up VTG as well as the ovary. We analyzed the VTG and VTG related proteins in the liver and testis of E2 treated male medaka by Western blotting. A band corresponding to VTG and several bands of smaller molecular weights were detected in the liver and testis samples. There were some differences in the band pattern between the two organs.

#### EXPRESSION OF STEROIDOGENIC ENZYMES IN THE QUAIL BURSA OF FABRICIUS

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The bursa of Fabricius (BF) provides the microenvironment for B-cell maturation. The BF produces the tripeptide hormone bursin, which induces phenotypic differentiation of avian B-lymphocyte precursor cells. Previously, we further identified the presence of  $\alpha$ -MSH and melatonin in the BF. However, the factors in the BF that promote B-lymphocyte maturation and/or mediate the endocrine effects of the organ are largely unknown.

Sex steroid hormones are synthesized from cholesterol by the sequential action of P450<sub>sc</sub>, 3 $\beta$ -HSD, P450<sub>c17</sub>, 17 $\beta$ -HSD and P450<sub>arom</sub> in the gonad, adrenal glands and the brain. To clarify the possibility of steroid hormone synthesis in the immune system, especially in the BF, and the mechanism of neuro-immuno-endocrine interactions, the present study investigated the expression of steroidogenic enzymes in the BF.

The expression of P450<sub>sc</sub>, 3 $\beta$ -HSD, P450<sub>c17</sub>, 17 $\beta$ -HSD and P450<sub>arom</sub> mRNA was detected by RT-PCR in the BF of Japanese quails (*Coturnix coturnix japonica*) aged 5 to 6 weeks. P450<sub>sc</sub>-immunoreactive cells were also found in the BF by the PAP method. These results suggest that steroid hormone synthesis may occur in the quail BF.

#### HYPO-ACTIVITY OF LIVER INSULIN RECEPTOR IN GOTO-KAKISAKI RATS IMPROVED BY NATURAL VANADIUM CONTAINING WATER

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For evaluating the prophylaxis treatment for diabetes mellitus, the effects of Mt. Fuji ground water containing natural vanadium on hypo-activity of insulin receptor of Goto-Kakizaki (GK) rats, a genetic model of NIDDM, were examined. Following the consecutive oral administration of vanadium containing water at the dose of 0.53  $\mu$ g/kg/day for 12 weeks, the levels of blood glucose, serum insulin and pancreatic insulin in GK rats were significantly improved. Furthermore, the alterations in the insulin receptor binding parameters and the levels of insulin receptor  $\beta$  subunit and primary insulin-like growth factor-I  $\beta$  measured by a radio receptor assay and western blotting, respectively, all recovered to their normal levels in Wistar rats. This observation may indicate the potentiation of cytosolic tyrosine kinase activity and the subsequent amelioration in the uptake of insulin in the liver by the vanadium-containing water administration, as previously reported for vanadium. These results suggest that the consecutive intake of relatively low concentrations of natural vanadium-containing water exerts anti-hyperglycemic effect by ameliorating the liver insulin receptor activity.

#### DEVELOPMENT OF THE PRIMARY CULTURE MODEL OF STROMAL CELLS IN MOUSE UTERUS AND VAGINA

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Effect of 17 $\beta$ -estradiol (E2) on uterine and vaginal epithelial (ep) cell proliferation could be mediated by stromal (st) cell-derived paracrine factors. To study the ep-st interactions in mice, we developed a primary culture model of st cells and examined cultured st cells in a response to E2. Uterine and vaginal st cells of immature mice were isolated using trypsin. To examine the effect of E2 *in vivo*, cells were grafted into the subrenal capsule of the host mice and grown for 2 weeks. Hosts were ovariectomized and treated with 5  $\mu$ g/kg E2 once. The BrdU labeling in cultured st cells was significantly increased by E2. To examine the effect of cultured st cells on ep cell proliferation, uterine and vaginal epithelia of adult mice were separated and recombined with cultured st cells. The recombinants were grafted and grown under the renal capsule of hosts for 3 weeks. The ep cells recombined with cultured st cells proliferated under the influence of hormonal environment of hosts, suggesting that cultured st cells still have the ability to stimulate ep cell proliferation. In conclusion, this culture system is useful to investigate uterine and vaginal ep-st interaction.

#### DEVELOPMENTAL EFFECTS OF ETHYNYL ESTRADIOL(EE2) ON REPRODUCTIVE ORGANS IN FEMALE MICE

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Reproductive organs can be developed abnormally in the female mice that were exposed to estrogen or estrogen-like substances perinatally. In the present study, effects of EE<sub>2</sub>, a synthetic estrogen, given by daily injections during gestational days 10-18 were examined in C57BL/6J mice. Fetuses from the mothers exposed to high doses of EE<sub>2</sub> or DES showed a low survival rate. Body weights of alive offsprings were less than those of the oil-exposed mice. The number of fetuses per litter was similar among all groups. At 30 days of age, polyovular follicles (PF) were found in the ovaries of all groups. Mice exposed to high doses of EE<sub>2</sub> showed a high incidence of PF. Moreover, the number of oocytes in a follicle showed a slight increase in a dose-dependent manner. At 40 days, vaginal epithelia of some of the EE<sub>2</sub>-exposed ovariectomized mice showed ovary-independent stratification and cornification. The number of BrdU-labeled cells in the vaginal epithelium was increased in a dose-dependent manner. These results showed the prenatal exposure to EE<sub>2</sub> or DES induces reproductive abnormalities, including PF, ovary-independent vaginal stratification and cornification.

#### AN INCREASE OF THYROXINE 5'-DEIODINASE ACTIVITIES IN VARIOUS TISSUES OF CHIPMUNKS DURING A HIBERNATION CONDITION

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Our previous study demonstrated that in rodents, hibernators had high activities of thyroxine 5'-deiodinases. Additionally, in chipmunks during hibernation,