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Abstracts

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ZEBRA FISH (*DANIO RERIO*) IN NEUROBEHAVIORAL RESEARCH: NEW MODELS, NEW APPLICATIONS, NEW CHALLENGES

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Introduction: Traditionally, zebrafish (*Danio rerio*) have been used in genetic and developmental bioscience research. More recently, zebrafish have been employed as a behavioral model to study stress and affective disorders, as well as neurodegenerative and many other disorders. Several models using experimental and/or genetic manipulations have been established to screen which factors are involved in precipitation of abnormal phenotypes. It is critical to note here that the neurophysiology of zebrafish is highly conserved, allowing physiological data to be reliably translated to further clinical research of psychiatric disorders. In addition, zebrafish are largely genetically analogous to humans, making this species an efficacious screen for genetic influence on aberrant behavior.

Methods: The novel tank diving test is the most commonly used experimental model of stress and anxiety in zebrafish, as it is simple, fast, and produces robust responses. As the novel tank *per se* is a mild stressor, it represents a good and valid experimental model of anxiety, affording a quick and reliable screen for anxiety-active drugs. Other behavioral models, such as place preference, spatial alternation, and T-maze, assess learning and memory in zebrafish using associative conditioning. These models have been effective in determining genetic factors involved in the cognitive deficits associated with neurodegenerative disorders, such as Alzheimer's and Huntington's disease. Transgenic zebrafish strains can also be used in these models to better assess the role of target genes in neurological disorders. Molecular biology techniques (e.g., PCR, HPLC, micro-array) illuminate differential gene expression, inter- and intracellular composition and other physiological markers that can be correlated with behavioral data, and then used to advance diagnostic criteria, prevention, and treatment of psychiatric disorders.

Results and Discussion: Experimental models based on the novel tank paradigm have produced robust anxiety-like responses that are highly sensitive to drug manipulation. Physiological analyses also provide support for behavioral data reported in this paradigm. For example, neuroendocrine responses (e.g., cortisol) reflect the anxiolytic or anxiogenic effects observed. Similarly, assessment of genetic and epigenetic factors can elucidate target genes involved in aberrant behavioral phenotypes. Also, zebrafish learning and memory paradigms have been useful in screening for cognitive impairment associated with neurodegenerative diseases, and identifying genetic mechanisms linked to pathogenesis of such disorders. Moreover, these models can be useful in assessing the genetic and physiological mechanisms linked to reward and drug dependence.

Conclusion: Zebrafish provide a high throughput animal model useful in behavioral neuroscience research. Several zebrafish behavioral models are effective in assessing abnormal phenotypes associated with brain disorders. These models will prove useful for analyzing the neurophysiology of stress, abnormal behavior, and neurological disorders. Further, experimental and transgenic models using zebrafish can provide an effective tool to identify genetic factors involved in pathogenesis, in addition to assessing the efficacy of drug treatments. Supported by NARSAD YI Award, Georgetown University Stress Physiology and Research Center (SPaRC) and Tulane University Intramural Research Funds.

COMPLEX, NATURAL, AND STEREOTYPIC BEHAVIORS, BEHAVIOR REGOGNITION AND TRANSLATIONAL RESEARCH

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Introduction: Behavioral researchers today are frequently faced with the challenges of finding adequate methods to observe and analyze animal behavior accurately, automatically, and in high throughput. Neurological diseases are being characterized by the behavioral phenotypes of mouse models and targeted mutations of genes expressed in the brain are revealing the underlying mechanisms of behavior. As a result the most comprehensive maps of the brain include molecular, cellular, system, and behavioral data. These dynamic, interactive, interdependent, and complex processes are reflected in the complex, natural, and stereotypic behaviors of lab animals.

Methods: Based on the theory of computer vision, artificial intelligence, and image processing, Behavior Recognition technology utilizes the information of an animal's full body, automatically identifies its important body parts, measures the movements of the body parts, together with other techniques like temporal-sequence analysis, automatically determines WHAT the animal is doing, establishing a completely novel and new technological framework in lab animal behavior analysis.

Discussion: This framework provides the capacity to automatically detect the complex, natural, and stereotypic behaviors in lab animals, answering the ever-increasing demand and challenges that arise from neuroscience research, drug discovery, genetic research, and other science areas. Integrating and synchronizing behavioral analysis with physiological measurements is embedded in this framework. Behavior Recognition technology has been proved sound and effective and has been successfully applied in many research areas.

Conclusion: Not only has Behavior Recognition technology brought about radical improvements in automated lab animal body behavior analysis, but has also provided great opportunity for supporting translational research since the same technological foundation can be equally applied to human body behavior research. Expanding on this foundation to develop technology capable of recognizing animal facial expressions as well as human facial expressions is an important future direction of research.

CROSS-FOSTERING EFFECT ON ACOUSTIC STARTLE REFLEX IN NORWAY GRAY RATS SELECTED FOR ELIMINATION AND FOR ENHANCEMENT OF AGGRESSIVENESS TOWARDS HUMANS

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Introduction: The aim of study was to examine the role of the maternal environment on the acoustic startle reflex and additionally assess the differences between two separate rat lines using the cross-fostering paradigm. The experiment was carried out on 48 male and 56 female Norway gray rats selected for elimination and for enhancement of aggressiveness towards human.

Methods: 1) Cross-fostering procedure: during the first day after delivery, pups were exchanged, i.e. pups born to a tame female were detached from her and transferred to an aggressive and *vice versa*. Control pups were nursed by their biological mothers. 2) The acoustic startle reflex amplitudes were measured at the age of 4 months in a SR-Pilot system (San Diego Instruments, San Diego, CA) as described (Popova et al., 1999). Ten acoustic stimuli were presented with an approximately 15-20 s rest interval.

Results: A repeated measures ANOVA revealed a significant effect of line [$F(1,84) = 82.65$, $p<0.001$] and trials [$F(9,756) = 43.81$, $p<0.001$] as well as significant line x trials interaction [$F(9,756) = 10.17$, $p<0.001$]. The line x trials x fostering interaction was marginally significant [$F(9,756) = 1.76$, $p=0.07$]. There were no significant effects of sex and fostering. Comparisons of individual startle response trials showed that the acoustic startle amplitude was significantly reduced in tame offspring compared with aggressive ones. A separate 2-way ANOVA showed the significant effect of sex [$F(1,40) = 20.74$, $p<0.001$] in the control tame rats only. The startle response amplitude for tame males was significantly higher than for the tame females. The averaged values of the startle reflex amplitude for 10 presentations of acoustic stimuli are also greater in the tame males than in females.

Conclusion: The finding that the amplitude of the startle response is higher in the aggressive than in the tame male rats (Popova et al., 2000) was supported by our current results. It proved that cross-fostering had no effect on the startle reflex amplitude in the aggressive males and females and in the tame females. The repeated presentation of ten startling auditory stimuli was also without cross-fostering effect in the GC (genetic cataleptics) and Wistar rat lines, which differed considerably from each in the startle response magnitude (Amstislavskii et al., 2000). This is at variance with the data on the role of postnatal maternal effects on the startle reflex amplitude. It is pertinent to note that the fostering of tame males by an aggressive mother affected the habituation of the startle reflex to presented auditory stimuli. Taken together, our data demonstrate that the differences in fear and/or anxiety between the tame and the aggressive rat lines, as estimated by the startle reflex amplitude, is largely genotype-dependent and hardly related to maternal influence.

EFFECT OF TERMINAL FRAGMENT OF MOUSE CHROMOSOME 13 ON CATALEPSY, ANXIETY, AGGRESSIVE AND DEPRESSIVE-LIKE BEHAVIOR

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Introduction: Catalepsy is a natural defensive reaction and is found in all vertebrates. At that same time, an exaggerated form of catalepsy is a syndrome of such severe disease as schizophrenia. Hereditary catalepsy was shown to be associated with depressive-like features in rats and mice. The QTL analysis showed that the major gene of catalepsy is located on the fragment of chromosome 13 between 47- to 75-cM. Congenic mice created using a genome fragment transfer are powerful models for studying the molecular mechanisms of behavior. The main aim was investigation the association of terminal fragment of mouse chromosome 13 with different form of defensive behavior using congenic lines.

Methods: The mapping of the major gene of catalepsy on chromosome 13 was accomplished by comparison of the catalepsy percentage in the congenic lines AKR.CBAD13Mit76C, AKR.CBA-D13Mit76A and AKR.CBA-D13Mit78 carrying the 59- to 70-, 61- to 70- and 71- to 75-cM fragments of chromosome 13 transferred from the CBA to the AKR genome. Catalepsy is estimated by the time during which an animal maintained the immobile posture on parallel bars, with the forepaws at a 45° angle above the hind legs. The genotypes of congenic mice are studied by means of PCR method with specific primers for microsatellites D13Mit76, D13Mit74, D13Mit78 and D13Mit214. Behavior was tested in open field, forced swim and intermale aggression tests with standard procedures.

Results and discussion: Catalepsy was found only in the AKR.CBA-D13Mit76C (52%) and AKR.CBA-D13Mit76A (52%) mice. In the open field test there was a decrease of exploratory behavior in the AKR.CBA-D13Mit76C and AKR.CBA-D13Mit76A mice: they did less rearing then AKR mice. There were no differences in anxiety behavior between these mice and AKR (number of enters in to the center, time spent in the center, and locomotor activity). It was shown that intermale aggression in the AKR.CBA-D13Mit76C and AKR.CBA-D13Mit76A (about 71%) mice became higher than in parent strain AKR (52%). No differences in immobility time were found in the forced swim test (FST) between these mice and AKR. The second (71-75cM) fragment of chromosome 13 had no effect on catalepsy, intermale aggression and behavior in the open field and FST in mice of congenic AKR.CBA-D13Mit78 line.

Conclusion: These results of testing mice in the open field test and FST indicate that the studied fragments of chromosome 13 do not contain genes regulating anxiety behavior and immobility time. Because aggression became higher in the AKR.CBA-D13Mit76C and AKR.CBA-D13Mit76A it is probable that in the AKR genome exits an unknown regulator that modifies function of genes in 61-70 cM fragment of chromosome 13 derived from CBA.

CHRONIC STRESS AFFECTS EXPRESSION OF TRANSCRIPTION FACTORS INVOLVED IN REGULATION OF PLASTICITY AND APOPTOSIS IN RAT HIPPOCAMPUS

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Introduction: Successful adaptation to stress involves inhibitory feedback at the level of hypothalamic-pituitary-adrenal (HPA) axis provided by the glucocorticoid receptor (GR), a steroid-dependent transcription factor (TF) which is very abundant in the hippocampus (HIPPO). Another transcription factor, nuclear factor κB (NFκB), considered as important stress sensor, has recently been implicated in synaptic plasticity, and some studies indicate its cytoprotective effect. There are numerous genes regulated by brain NFκB, including neural cell adhesion molecules (such as NCAM and L1) and genes that encode proteins involved in apoptosis (like *bax* and *bcl-2*). We investigated effects of chronic social isolation on the expression level and compartmental distribution of GR and NFκB transcription factors in the hippocampus, and in parallel, changes in expression of GR, NFκB, NCAM, L1, and *bax* and *bcl-2* genes, potentially regulated by these TFs.

Methods: Serum corticosterone was determined by ELISA assay, protein expression by Western blot followed by densitometric quantification and mRNA expression by RT-PCR.

Results and Discussion: Even though we did not detect compartmental redistribution of GR protein in HIPPO, at the level of mRNA, GR was down-regulated, indicating possible involvement of some other signaling pathway in its regulation. On the other hand, NFκB showed translocation from cytoplasm to the nucleus which was in accordance with its elevated mRNA level under chronic stress. In addition, NCAM gene was up-regulated possibly through NFκB activation, while L1 remained unchanged. Interestingly, both *bax* and *bcl-2* genes were down-regulated but *bax/bcl-2* mRNA ratio remained unaffected by chronic stress.

Conclusion: The observed translocation of NFκB to the nucleus and concomitant up-regulation of NCAM gene could be a part of compensatory neuroprotective response to the possible chronic stress induced damage. This is accompanied by blunted corticosterone response to chronic stress followed by lack of GR translocation to the nucleus.

CHRONIC PSYCHOSOCIAL STRESS MAY ALTER ANTIOXIDANT ENZYME CAPACITY IN PREFRONTAL CORTEX OF WISTAR RAT BRAIN

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Introduction: The hypothalamic-pitutary-adrenal (HPA) axis plays a primary physiological role in response to stress, exerting negative feedback through glucocorticoids, on several brain structures, including prefrontal cortex (PFC). It is well known that intensive stress response results in the production of ROS, *i.e.* superoxide anion radical ($O2^{\cdot-}$), hydroxyl radical ($HO^{\cdot}$) and hydrogenperoxide (H_2O_2). Brain is considered to be particulary susceptibile to oxidative stress, since it is enriched in peroxidizable fatty acids, and since it consumes about 20% of the total oxygen consumption for its relatively small weight (2%) and is not particularly enriched in antioxidant defenses. The aim of this study was to determine the effects of chronic 21 day isolation stress on antioxidant enzymes (AOEs) expression in Wistar rat brain.

Methods: Serum corticosterone (CORT) was determined by ELISA assay and blood glucose (GLU) level was determined by Accutrend strips. CuZn-superoxide dismutase (CuZnSOD), Mn-superoxide dismutase (MnSOD), catalase (CAT) and glutathione peroxidase (GPx) protein expression were assayed by Western blot, followed by densitometric quantification.

Results and discussion: In terms of chronic social isolation we detected decreased CORT level, as well as, low GLU level. Since increased AOEs are well known indicators of elevated ROS, we measured their protein expression, and quantification showed the elevation of cytosolic CuZnSOD and CAT expression, whereas MnSOD and GPx expression remained unaffected. Taking into account poor CAT activity in the brain, the question arises whether its elevation is enough for detoxification of increased H_2O_2 production due to elevated CuZnSOD, since the main scavenger of H_2O_2, GPx, remained unaltered.

Conclusion: Our results suggest that the state of oxidative stress may exist in rat prefrontal cortex under social isolation and low CORT and GLU conditions, and that the detoxification of generated ROS may not be efficient, thus compromising negative feedback at the level of PFC.

INFLUENCE OF ESTROUS CYCLE ON EXPLORATIVE BEHAVIOUR OF WILD-TYPE AND PRODYNORPHIN KNOCKOUT MICE

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Introduction: For several years dynorphin, a member of the opioid peptide family was suggested to play a regulatory role in numerous functional pathways of the brain. In line with its localization in hippocampus, amygdala, hypothalamus and striatum these functions resemble learning and memory, emotional control and stress response. Male prodynorphin deficient (Dyn KO) mice display an anxiolytic phenotype (Wittmann et al, 2008). However, emotional control and stress response depend on the hormonal state and differ between sexes.

Methods: We analyzed anxiety and stress related behaviour in correlation to the estrous cycle in female wild-type (WT) and Dyn KO mice.

Results and Discussion: In the elevated plus maze test Dyn KO mice showed a significant anxiolytic phenotype with about double time spent, distance travelled and entries in the open arm at all estrous stages compared to WT mice. In addition, WT mice showed a significant increase in anxiety related parameters during proestrous and estrous. This increased anxiety was markedly attenuated by prodynorphin deficiency without interference on total activity. In the open field test WT mice showed significantly increased anxiety during the estrous stage. This effect was abolished in Dyn KO mice. In the light dark test WT mice showed a decrease in time spent and distance travelled in the lit area during proestrous. In contrast, the behaviour of KO mice was not altered throughout the stages. In the tail suspension test Dyn KO mice spent comparable times immobile throughout the estrous stages. WT mice showed a significant increase (by ca. 40%) in immobility during diestrous. In the forced swim test proestrous WT mice showed similar decreases in time spent immobile, during the initial and final 4 minutes of a 15 minutes trial. At the beginning of the trial, when anxiety induces escape behaviour, Dyn KO mice showed less activity. In contrast, in the last four minutes, which reflects stress induced depression like behaviour WT mice were more immobile.

Conclusion: Our data support the influence of estrous stage on anxiety in female mice. Of note is the fact that the influence of the estrous stage appears to be abolished by the prodynorphin deficiency. This is in line with altered stress responses during the estrous cycle in WT, but not in Dyn KO mice. The functional and pharmacological background of the interplay of hormones and dynorphin will be investigated in further experiments.

PHEROMONAL STRESS IN DYSTROPHIN-DEFICIENT MICE AND ITS EFFECT ON SEXUAL BEHAVIOUR

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Introduction: It had been clearly established by past researches that 2,5-dimethylpyrazine (2,5-DMP), a murine pheromone, produced by females under the conditions of excessive population density, can be recognized as a stressor. It induces chromosome aberrations and other mitotic and meiotic disorders in both somatic cells and gametes; and suppresses the reproductive system by an unknown mechanism. We hypothesized that the use of a mouse strain with cytoskeleton abnormalities can shed light on mechanisms underlying process of stress-induced chromosome damaging, as microtube apparatus plays an important role in chromosome segregation and cell division. Therefore a strain with defected membrane protein dystrophin has been chosen for this study (C57BL/*mdx*). As behavior response induced by 2,5-DMP is still yet to be studied and given the specifics of this pheromone, social behavior of mice has been investigated.

Methods: To evaluate the impact of pheromonal stress on chromosomal apparatus during cell division mitotic disorders frequency in bone marrow cells was measured in 17 days after 24 hours treatment with 2,5-DMP. To investigate how 2,5-DMP effects social behavior, mice were subjected to pheromone exposure during 17 days and a modified intruder-resident test (with either male or female in estrus as an intruder depending on the test day, and intruder being placed inside pellucid perforated plastic box) was performed on different days.

Results and Discussion: Cytogenetic effect of 2,5-DMP: 24 hours exposure to 2,5-DMP significantly increased frequency of mitotic disorders in bone marrow cells of C57BL/*mdx* male mice from 4,6% in control group to 10,4% in stressed animals. The spontaneous level of mitotic disorders was comparatively not very high. Taken together the data other studies previously had shown, our results suggest that bone marrow cells of C57BL/*mdx* mice are more sensitive to 2,5-DMP-induced stress then cells of other strains. Behavioral effect of 2,5-DMP: When presented a female in estrus, social activity of male CBA mice, compared to that when presented a male mice, increased in control group: a significant difference of duration and number of contacts were detected. However no significant difference was observed in stressed group.

Conclusion: Evidence of 2,5-DMP showing chromosome damaging activity has been documented, this time for C57BL/*mdx* mice. Furthermore, this strain has shown itself to be more sensitive to pheromonal exposure then other strains, suggesting that cytoskeleton, and dystrophin in particular, might be a channel through which 2,5-DMP exerts its effects. Clearly, it requires further investigation. Alterations of social behavior, observed in stressed mice might be one of the ways of population density regulation. Supported by: RFBR № 08-04-01335

BEHAVIORAL EFFECTS OF TRANSGENIC MANIPULATION OF NEUROTROPHIN RECEPTOR TRKB SIGNALING

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Introduction: Recent studies have connected BDNF signaling through trkB receptor with mood disorders and the antidepressant response. We have performed a behavioral test battery in two mouse strains, overexpressing either the full-length neurotrophin receptor trkB (trkB.TK+) or the dominant negative truncated trkB.T1 isoform in postnatal neurons.

Methods: Male mice were behaviorally tested in the following paradigms: elevated plus-maze (EPM), light/dark exploration, open field, y-maze, beam walking, coat hanger, hot plate, rotarod, fear conditioning, forced swimming test (FST), water maze, pre-pulse inhibition and CLAMS (The Comprehensive laboratory animal monitoring system, physiology). Female mice were placed in an IntelliCage-devise in which mice can be detected individually and given different learning tasks by regulating individual water supply. Animals: TrkB.TK+ mice (male 14 WT and 14 TG, female 8 WT and 8 TG), TrkB.T1 mice (male 11 WT and 12 TG, female 8 WT and 8 TG). The genetic background of both strains was C57BL/6.

Results and Discussion: Neither strain showed a clear phenotype in the test battery. In the EPM, TrkB.T1 animals showed decreased anxiety while they showed increased anxiety-related behavior in the fear conditioning test. TrkB.TK+ mice showed decreased baseline locomotor activity in the fear conditioning test while the freezing score was equal to the WT mice. In the water maze test no differences in learning rate could be observed, but trkB.T1 and trkB.TK+ mice showed decreased and increased swimming speed, respectively. In the FST trkB.T1 mice showed increased immobility, while trkB.TK+ mice showed no difference when compared to the WT mice. In the IntelliCage study both strains showed decreased activity and increased latency to visit drinking corners during the first seven hours. This might indicate increased anxiety-like behavior. There were no differences in learning tasks in the IntelliCage.

Conclusion: Both strains trkB.TK+ and trkB.T1 showed similar results in the IntelliCage. TrkB.TK+ mice showed no clear anxiety-related phenotype while trkB.T1 mice had opposite results from different anxiety-related tests. These strains have different swimming speed in the water maze, but it doesn't seem to influence their learning abilities. The results with the trkB.TK+ strain differ substantially from previous results obtained with mice from a mixed background. It is possible that backcrossing has influenced transgene expression or the effect of the background strain is stronger than the effect of mutation.

THE ROLE OF NOVEL CELL ADHESION MOLECULE, NECTIN1, IN NEURITE OUTGROWTH AND NEURON SURVIVAL

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Introduction: Neurons communicate and store information by changes in the architecture and strength of their synapses, and neural cell adhesion molecules are probably among the most important regulators of the morphology and function of synapses in the CNS. Nectins are Ca^{2+} independent cell adhesion molecules expressed in many tissues including the nervous system, and comprise a family of a least four members – nectin1-4. All members of the nectin family have an extracellular region containing three Ig-like domains, the first and second Ig-like domain of Nectin1 are hypothesized to be necessary for the formation of a cis-dimer, but the function of the third Ig-like domain is currently unknown. The aim of the study was to test if the function of the third Ig module of Nectin1 (Nec1_Ig3) has a neuroprotective effect, and whether it induces neurite outgrowth.

Methods: Primary cultures of cerebella granule neurons (CGNs): CGNs were isolated from the cerebellum of post-natal 7-8 days old rat-pups (*Wistar rats*) and incubated for 24 hours with different concentrations of the Nec1_Ig3 module and derived peptides p1 and p2 to evaluate neurite outgrowths. Survival effect of the peptide and module was estimated by incubating CGNs for 7 days, subsequently inducing apoptosis by lowering KCl concentration in the media and adding different concentrations of the peptides and the module to evaluate a possible protective effect against apoptosis. The *Pichia pastoris* expression system: A yeast expression system capable of producing recombinant proteins was used to express the third Ig module of Nectin1.
Surface Plasmon Resonance (SPR): A method for real-time investigation of protein-protein interactions was used to test binding between a variety of receptor tyrosine kinases to the third Ig module of Nectin1.

Results: The two peptides derived from the Ig3 module of Nectin1, p1 and p2 and the module itself were shown to induce neurite outgrowths and protect against apoptosis in CGN. The module and the p2 were shown to bind FGFR1 and FGFR3 in SPR studies. It is currently being tested which of the receptor tyrosine kinases are involved in the induction of neurite outgrowths and survival of the module and the peptides.

Conclusion: The Ig3 module of Nectin1 is involved in neurite elongation and neuron survival, probably via interaction with a receptor tyrosin kinase.

MODELING SUBSTANCE WITHDRAWAL SYNDROME IN ZEBRAFISH (DANIO RERIO)

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Introduction: A widely used animal model in genetics and developmental biology, Zebrafish (*Danio rerio*) are now becoming popular in behavioral neuroscience research. Their behaviors are easily quantified, and the effects of pharmacological intervention are robust and almost immediately evident. Moreover, physiological and genomic data are easily obtained, facilitating analysis using proven molecular biology techniques. This allows researchers to examine the neurological foundations of a behavior or disease across multiple levels of analysis, from genetics to physiology to brain and behavior, with an ease not found in other common animal models. In these studies, we examined the behavioral and physiological characteristics of withdrawal from ethanol (EtOH), diazepam and morphine in zebrafish.

Methods: To examine the effects of EtOH withdrawal, zebrafish were exposed to EtOH (0.3% vol/vol) for 8 days. On the 9th day, zebrafish were moved into a new fresh water tank and remained there 12 h prior to behavioral phenotyping in the Novel Tank Exposure Test. In zebrafish chronically exposed to diazepam (5 μM) for two weeks, the withdrawal was induced by placing them in a new fresh water tank for 3 days prior to testing in the novel tank. To examine acute morphine withdrawal, morphine (2 mg/L) was administered for 1 week. These fish were then placed in naloxone (1 mg/L) for 1 h before behavioral testing in the novel tank. Control animals were subjected to identical experimental procedures in absence of the pharmacological treatments. Immediately following removal from the novel tank, zebrafish were euthanized in a tricaine solution (0.2 mg/L), and all bodies were homogenized for cortisol extraction. Cortisol was isolated with two 5-ml ether washes, transferring the ether into a glass tube after each wash. Once the ether had evaporated, cortisol was reconstituted in 1ml of 1X PBS and concentrations were quantified using a human salivary cortisol ELISA assay (Salimetrics LLC, PA, USA).

Results and Discussion: EtOH withdrawal evoked a clear anxiogenic response in zebrafish. Compared to control fish, the EtOH withdrawal group showed fewer transitions into the upper half of the novel tank ($p=0.0036$), more freezing bouts ($p=0.003$) and were frozen for a greater duration ($p=0.0012$). Cortisol measures support this interpretation, with a lower average concentration of cortisol in chronically treated EtOH fish (0.159 μg/dL) than in EtOH withdrawal fish (0.274 μg/dL; $p=0.013$). In a similar fashion, diazepam withdrawal had anxiogenic effects in zebrafish, as the withdrawal cohort made fewer transitions into the upper half ($p=0.011$), spent less time in the upper half ($p=0.004$), and displayed more erratic movements ($p=0.012$). However, obtained cortisol data was inconclusive, as diazepam control fish and diazepam withdrawal fish had statistically similar whole-body cortisol concentrations. Statistically significant difference was found for erratic movements, in which naloxone treated fish made on average 22.8$\pm$3.4 erratic movements during the 6-min interval and chronic morphine controls 9.16$\pm$1.8; $p=0.01$.

Conclusion: Within each of these designs, our data demonstrates that behavioral and physiological quantification of withdrawal signs can be easily performed in zebrafish. As expected in withdrawal from CNS depressants, the cessation of chronic EtOH, diazepam and morphine treatments induced anxiogenic behaviors and physiological responses in zebrafish. The average sample size for groups represented here was ~10-20 fish per group for behavioral testing and approximately 7-10 fish in cortisol extraction. In the future, we plan to further examine withdrawal behaviors in zebrafish, with an emphasis on the use of larger sample sizes, and a wider spectrum of drugs. However, the unique potential and utility of *Danio rerio* as a model for behavioral neuroscience and drug use/abuse research is becoming increasingly evident. Supported by NARSAD YI Award, Georgetown University's Stress Physiology and Research Center (SPaRC), Tulane University and University of New Orleans Intramural Research Funds.

CB1 RECEPTORs AND MORPHINE WITHDRAWAL

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Introduction: Pharmacologically based evidence indicates that CB1 cannabinoid receptors (CB1Rs) are present in guinea pig ileum (GPI) and their activation reduces acetylcholine (Ach) release. Dependence can be induced and measured *in vitro* by using GPI. The contraction due to opioid withdrawal is caused by acetylcholine release.

Methods: Synthesized molecules that act on the CB1Rs are widely studied and the large availability of CB1Rs agonists and antagonists provides powerful tools to determine the role of these receptors in mediating some of physiological and pharmacological effects in the myenteric neurones.Given the relationship between CB1Rs/Opioid Withdrawal/Ach system, in the present paper we have designed six new CB1Rs agonists named A-F and evaluated their role in mediating morphine withdrawal in GPI. Also, a comparative study was performed by using the CB1Rs synthetic cannabinoid WIN 55,212-2 and CP 55,940.

Results and Discussion: The results of our experiments indicate that both WIN 55,212-2 and CP 55,940 ($1x10^{-8}$-$5x10^{-8}$-$1x10^{-7}$M) were able to reduce morphine withdrawal in a concentration-dependent manner. Very similar results were obtained with the new CB1Rs agonists (A-F) used at same concentrations.

Conclusion: Our experiments indicate that CB1Rs are involved in the control of morphine withdrawal *in vitro* thus confirming an important functional interaction between the cannabinoid and opioid system.

UNDERSTANDING ANXIOGENIC RESPONSES TO ENVIRONMENTAL MANIPULATIONS IN ZEBRA FISH (*DANIO RERIO*)

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Introduction: Zebrafish have attained a preeminent status as models of stress and anxiety, displaying robust anxiogenic responses to various stressful environmental manipulations. Here, we explored anxiety-like phenotypes, in order to better understand how zebrafish respond to stress, and further validate them as an experimental model of stress.

Methods: To evoke anxiety, zebrafish were exposed for 6 (acute) or 90 (long-term) min to 7-10 ml of pheromone solution generated from the damaged epidermal cells of sacrificed animals. In the predator exposure test, zebrafish were pre-exposed to their natural predator, the Indian Leaf Fish (*Nandus nandus*) for 20 min (acute stress) or 24-72 h (chronic exposure). In all experiments, zebrafish were observed individually in the novel tank test for established stress phenotypes.

Results and Discussion: Acute alarm pheromone produced robust anxiety (replicating the results of previous studies), such as fewer transitions to the upper half ($p<0.005$), less time in the upper half of the tank ($p<0.005$), and more erratic movements ($p<0.05$). Interestingly, long-term alarm pheromone exposure showed no significant differences between experimental and control subjects. Thus, alarm pheromone appears to only be effective in acute doses (most likely reflecting its natural use as a danger signal to nearby shoals), whereas long-term exposed fish become desensitized/habituated to the alarming effects of the pheromone. While acute predator exposure has been established as an anxiogenic paradigm in zebrafish, chronic exposure was a novel experiment yielding significant results. Both 24- and 72-h experiments reduced time spent on the bottom ($p<0.0005$), paralleled with a greatly reduced latency time to reach the upper half ($p<0.0005$ and $p<0.00005$ respectively). Unlike results from alarm pheromone and acute predator exposure test (provoking a protective diving/freezing responses), chronic predator exposure data imply avoidance of the bottom half of the tank, rather than the top. This suggests that zebrafish exposed to the Indian Leaf Fish attempt to escape the bottom half of the tank, where the predator was predominantly located during exposure. In line with this “anxiogenic” interpretation of these data, chronically exposed subjects also exhibited an increased number of erratic movements ($p<0.05$) in both 24- and 72-h tests. In general, our experiments elucidate the complex yet robust anxiety-related phenotypes of zebrafish. While top dwelling generally signifies lessened anxiety [through increased exploration], in the predator exposure test, it may alternatively indicate a strong avoidance. Therefore, several different domains must be carefully balanced in the observation and subsequent interpretation of zebrafish anxiety behavior, including 1) locomotion (overall activity), 2) reduction of exploration (anxiety), 3) avoidance, and 4) erratic movements (fear- and/or escape-like behavior). Only detailed analyses of these affected domains together may provide insight into the type of stressor being applied.

Conclusion: Alarm pheromone and predator exposure evoke strong anxiety responses in zebrafish, reaffirming the utility of this species in stress research. In addition, this model organism displayed stimuli-specific responses, indicating its notable ability to adapt its behavior to specific stressors. Supported by NARSAD YI Award, Georgetown University Stress Physiology and Research Center (SPaRC) and Tulane University Intramural Research Funds.

VITAMIN D AND SENSORY FUNCTIONS

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Introduction: Vitamin D (VD) is a fat-soluble seco-steroid hormone, which is synthesized in the skin (under the influence of ultraviolet light from the sun) or obtained from food. Metabolites of VD, 25-hydroxyvitamin (25(OH)D) and 1,25-dihydroxyvitamin D (1,25 $(OH)_2D$), have their effects through the vitamin D receptor (VDR), and alter the expression of specific target genes. VD is one of the major regulators of mineral homeostasis in mammals, and is essential for normal bone growth and mineralization. However, several recent studies have postulated functions for VD in the regulation of physiological brain functions (such as neuroprotection, regulation of behavior, as well as motor functions). Also, VDRs are expressed throughout the sensory system. Less is known about the role of vitamin D in the regulation of sensory functions, such as hearing, balance, olfactory, and gustatory functions.

Methods: We used vitamin D receptor knockout (VDR) "Tokyo" mice fed with a special rescue diet to normalize blood mineral level. Hearing of the mice was measured by auditory brain stem response (ABR). Rotarod, tilting box, rotating tube, and forced swimming test were used to analyze vestibular functions and motor performance. The buried food test was applied to analyze olfactory functions and the two-bottle "preference" test was used to study gustatory functions.

Results and Discussion: Hearing loss developed earlier in VDR mutant mice compared to wildtype controls. Also, VDR mutant mice showed shorter latency to fall in the rotarod test and demonstrated a smaller fall angel in the tilting box test. These mice showed mostly vertical swimming and sinking episodes in contrast to control wild type mice. Olfactory and gustatory functions were unaltered in VDR mutant mice.

Conclusion: Collectively, these data confirm that mutation of VDR in mice leads to altering hearing and vestibular phenotypes, but does not play a major role in olfactory and gustatory functions. These results support the role of the vitamin D/VDR system in the regulation of auditory and vestibular system, and may have clinical relevance, enabling a better focus on hearing loss and balance disorders associated with dysfunctions in the VD/VDR endocrine system.