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Differential regulation of catechol-O-methyltransferase expression in a mouse model of aggression

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Abstract

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This study was designed to understand molecular and cellular mechanisms underlying aggressive behaviors in mice exposed to repeated interactions in their home cage with conspecifics. A resident–intruder procedure was employed whereby two males were allowed to interact for 10 min trials, and aggressive and/or submissive behaviors (e.g., degree of attacking, biting, chasing, grooming, rearing, or upright posture) were assessed. Following 10 days of behavioral trials, brains were removed and dissected into specific regions including the cerebellum, frontal cortex, hippocampus, midbrain, pons, and striatum. Gene expression analysis was performed using real-time quantitative polymerase-chain reaction (qPCR) for catechol-*O*-methyltransferase (COMT) and tyrosine hydroxylase (TH). Compared to naive control mice, significant up regulation of COMT expression of residents was observed in the cerebellum, frontal cortex, hippocampus, midbrain, and striatum; in all of these brain regions the COMT expression of residents was also significantly higher than that of intruders. The intruders also had a significant down regulation (compared to naive control mice) within the hippocampus, indicating a selective decrease in COMT expression in the hippocampus of submissive subjects. Immunoblot analysis confirmed COMT up regulation in the midbrain and hippocampus of residents and down regulation in intruders. qPCR analysis of TH expression indicated significant up regulation in the midbrain of residents and concomitant down regulation in intruders. These findings implicate regionally- and behaviorally-specific regulation of COMT and TH expression in aggressive and submissive behaviors. Additional molecular and cellular characterization of COMT, TH, and other potential targets is warranted within this animal model of aggression.

Keywords

Hippocampus; Midbrain; qPCR; Resident–intruder; Social defeat; Tyrosine hydroxylase

Introduction

Aggression is defined as behavior in humans and animals with intent to deliver noxious stimulation and/or to act in a destructive manner (Volavka 2002). Aggression is a major societal problem worldwide, and poses serious strains on public welfare and criminal justice systems in the USA. Multiple pathways to aggressive behavior exist, but both human and animal model studies suggest that aggressive behaviors are frequently associated with alterations in catecholaminergic neurotransmission, and manipulation of catecholaminergic activity (particularly dopaminergic) can modulate aggressive responses. Aggressive behavior and violence are included in the physical expression of several psychiatric disorders including schizophrenia and manic-depressive disorder, among others (Miczek et al. 2002; Volavka 2002; Howard et al. 2010). Current treatments of aggressive behavior in these disorders include antipsychotic medications, among which clozapine has superior and specific antiaggressive efficacy (Citrome et al. 2004; Volavka 2006; Goedhard et al. 2006, 2010). Although a genetic component is hypothesized to contribute to the etiology of these complex neuropsychiatric disorders, both genetic as well as epigenetic events are likely to be factors that underlie disease pathogenesis and await further characterization using functional genomics approaches.

Two enzymes, catechol-*O*-methyltransferase (COMT) and monoamine oxidase (MAO) are responsible principally for catecholamine catabolism in the brain (Kopin 1994; Napolitano et al. 1995). COMT is abundant in brain, and is localized to neuronal and glial populations. The transcriptional activity of the genes encoding these enzymes is governed by common functional polymorphisms. For example, a common biallelic single nucleotide polymorphism (SNP) involving a methionine (met) to valine (val) substitution at codon 158 of the COMT gene has been localized to chromosome 22q11.1–q11.2 (Grossman et al. 1992). The met allele is associated with lower COMT enzymatic activity, whereas the val

allele is associated with higher activity. Subjects homozygous for the met allele have a three- to fourfold reduction in COMT activity relative to subjects homozygous for the val allele, with heterozygous subjects demonstrating intermediate levels of enzymatic activity.

It is likely that COMT gene expression levels affect COMT enzymatic activity in response to environmental contingencies, and effectively modulate aggressive behavior over time. Alterations in COMT gene expression have been studied in aggressive mice by a Russian group (Kudryavtseva et al. 2004), which drew our attention to this phenomenon. These authors have also observed increased tyrosine hydroxylase (TH) gene expression in related mouse aggression paradigms (Kudryavtseva et al. 2004; Filipenko et al. 2001). Accordingly, we have included both COMT and TH gene expression assays as part of the present study.

Behavioral and physiological changes caused by paradigms that elicit aggressive and/or stressful social defeat responses in animal models are typically maintained for a significant amount time following the end of the experimental trials (Buwalda et al. 2005). The molecular and cellular underpinnings of neuroadaptive responses responsible for initiating and sustaining these alterations remain unknown and of high importance. This study was designed to gain insight into molecular changes induced in different brain regions following aggressive and submissive behaviors expressed in adult male mice. Several regions were examined that contain catecholaminergic nuclei (e.g., midbrain and pons) as well as projection sites that receive significant catecholaminergic terminal input including the cerebellum, frontal cortex, hippocampus, and striatum (Fig. 1).

A goal is to understand the molecular and cellular mechanisms that underlie aggressive behaviors in mice exposed to repeated aggressive interactions with conspecifics. A resident–intruder paradigm (where a resident rodent executes aggressive behaviors against an intruder rodent) is a well-known model of aggressive activity (Miczek et al. 1994; Filipenko et al. 2001; Johnson et al. 2003; Bartolomucci et al. 2003; Velez et al. 2010), and was employed in the current study. Adult male mice were used throughout the study, as the previously reported association between COMT levels and aggression in mice was limited to males (Gogos et al. 1998).

Methods

Resident–intruder protocol

Adult male C57BL/6 mice ($n = 88$; 20–30 g, Taconic, Germantown, NY) were initially housed individually for 5 days with a standard 12:12 h light/dark cycle and ad libitum access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Nathan Kline Institute/NYU Langone Medical Center. A resident–intruder test procedure was employed (Filipenko et al. 2001; Bartolomucci et al. 2003; Velez et al. 2010). Briefly, 5 cohorts of 10–12 animals of approximately the same weight were placed in pairs in large plastic cages ($15 \times 9 \times 7$ in.) divided in half with an opaque plastic partition for 2 days (Supplemental Fig. 1). Five mice per cohort were maintained throughout the trial period in individual cages to serve as controls. After 2 days of adaptation, testing was started during the light-cycle by removal of the partition and antagonistic behavior observed and recorded for a 10 min period. A behavioral expert assessed aggressive and/or defensive behaviors (degree of attacking, biting, chasing, grooming, rearing, and/or upright posture). Animals were rated on an aggressive behavioral scale of 1–5, with 1 being the most aggressive and 5 being the least aggressive, based upon modifications of the procedures employed by Filipenko et al. (2001). The more aggressive mouse of each pair was considered a winner, and was returned to its homecage (deemed the resident mouse) (Filipenko et al. 2001; Kudryavtseva et al. 2004). The submissive mouse of the pair was considered the loser (deemed the intruder mouse) and paired with another

dominant opponent in an unfamiliar cage (Filipenko et al. 2001; Kudryavtseva et al. 2004). If an aggressive confrontation lasted more than 3 min, resident and intruders were separated by the partition. Behavioral testing was repeated once daily for 10 days. The test procedure yielded half of the subjects as residents (aggressive) and half of the subjects as intruders (submissive). To reiterate, resident and intruder status was not wholly designated on the first encounter, rather the resident–intruder encounters were repeated for 10 trials on consecutive days. Thus, any mouse may be designated as a resident on one trial and subsequently be designated as an intruder on the next consecutive trial. This procedure enables the more aggressive mice to become readily apparent, typically by trials 4–5.

After the last daily test procedure, half of the cohort (residents and intruders) were killed by decapitation 10 min following the behavioral trial and the second half of the cohort was killed 24 h later. The total number of mice utilized for qPCR and immunoblotting included residents ($n = 30$), intruders ($n = 33$), and controls ($n = 25$). Brains were removed rapidly using a scalpel and a micropunch and frozen on dry ice as described previously (Javitt et al. 2005; Ginsberg 2005a; Ginsberg et al. 1995, 1996), with the aid of a mouse brain atlas (Paxinos and Franklin 2001) and the C57BL/6J virtual atlas web page (Williams et al. 1999) for orientation. Dissected regions including the cerebellum, frontal cortex, hippocampus, midbrain, pons, and striatum were placed in microfuge tubes and stored at -80°C until use. The frontal cortex dissection included gray matter from gustatory and motor-sensory regions. Striatal dissections contained the caudoputamen but not the nucleus accumbens or globus pallidus. The hippocampal dissection included portions of the CA1 and CA3 regions as well as the dentate gyrus, but not the hilar area. The midbrain dissections centered around the substantia nigra (pars compacta and pars reticulata) region with minimal inclusion of the ventral tegmental area or cerebral peduncle. A representative anatomical schematic of the six microdissected areas is illustrated in Fig. 1.

Gene expression assessments

RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) and chloroform, precipitated utilizing isopropanol, and resuspended in 18.2 MΩ RNase-free water (Nanopure Diamond, Dubuque, IA). RNA purity and concentration was assessed via bioanalysis (2100, Agilent Technologies, Santa Clara, CA) (Ginsberg 2005b; Alldred et al. 2009). Real-time quantitative PCR (qPCR) was performed on microdissected frozen tissue samples (Fig. 1) as described previously (Ginsberg 2005b; Alldred et al. 2008, 2009; Jiang et al. 2010). TaqMan hydrolysis probes designed against COMT (Mn00514377_m1), TH (Mm00447557_m1), and the housekeeping gene glyceraldehyde-3 phosphate dehydrogenase (GAPDH; mM99999915-g1) were purchased from Applied Biosystems (ABI, Foster City, CA). Samples were run on a real-time PCR cycler (7900HT, ABI) as per the manufacturer's instructions. Standard curves and cycle threshold (Ct) were generated using standards obtained from total mouse brain RNA. The ddCT method was employed to determine relative gene level differences with GAPDH qPCR products used as a control (ABI 2004; Alldred et al. 2009). A total of 10–16 independent samples per region for residents, intruders, and controls were assayed via qPCR, and averaged to obtain COMT and TH expression levels for each brain region.

Immunoblotting

Microdissected tissue samples ($n = 5$ residents at 24 h post test procedure, $n = 7$ intruders at 24 h post test procedure, and $n = 4$ naive controls) from the hippocampus and midbrain were homogenized in a 20 mM Tris–HCl (pH 7.4) buffer containing 10% (w/v) sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA), 5 mM ethylene glycol-bis(β -aminoethylether)- N,N,N',N' -tetra-acetic acid (EGTA), 2 mg/ml of the following: (aprotinin, leupeptin, and chymostatin), 1 mg/ml of the following: [pepstatin A, antipain, benzamidine, and

phenylmethylsulfonyl fluoride (PMSF)], 100 µg/ml of the following: [soybean trypsin inhibitor, Na-*p*-tosyl-L-lysine chloromethyl ketone (TLCK), and *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK)], 1 mM of the following: (sodium fluoride and sodium orthovanadate) and centrifuged as described previously (Ginsberg 2010). All protease inhibitors were purchased from Sigma (St. Louis, MO). Identical amounts of proteins (10 µg) were loaded into a gel electrophoresis apparatus, subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE; 4–15% gradient acrylamide gels; Bio-Rad, Hercules, CA), and transferred to nitrocellulose by electroblotting (Mini Transblot, Bio-Rad). Nitrocellulose membranes were blocked in blocking buffer (LiCor, Lincoln, NE) for 1 h at 4°C prior to being incubated with well-established monoclonal antibodies directed against COMT (611970; BD Biosciences, Chicago, IL; 1:1,000 dilution; technical datasheet weblink; http://www.bdbiosciences.com/external_files/pm/doc/tds/tl/live/web_enabled/C16120_611970.pdf) and β -tubulin (T5293; TUBB; Sigma, 1:1,000 dilution; technical data-sheet weblink; <http://www.sigmaaldrich.com/etc/medialib/docs/Sigma/Datasheet/t5293dat.Par.0001.File.tmp/t5293dat.pdf>) in blocking buffer overnight at 4°C. Membranes were developed affinity-purified secondary antibodies conjugated to IRDye 800 (Rockland, Gilbertsville, PA) and visualized using an infrared detection system (Odyssey, LiCor). Following the assay of COMT immunoreactive bands, the immunoblots were stripped and reprobed with the TUBB antibody. Immunoblots were quantified by densitometric software supplied with the instrument. Signal intensity of immunoreactive bands was normalized to TUBB immunoreactivity for each assay (Ginsberg 2005a, 2010).

Statistical analysis

Residents, intruders, and controls were compared with respect to two outcomes: COMT and TH gene expression levels. The comparisons at all six pre-specified areas of the brain were performed simultaneously. This was done by modeling the outcome (COMT or TH expression) as a function of region (a factor with six levels: cerebellum, frontal cortex, hippocampus, midbrain, pons, and striatum), time from behavioral test to sacrificing the animal (a factor with two levels: 10 min and 24 h), and group (a factor with three levels: residents, intruders, and controls), and all 2- and 3-way interactions. To account for the correlation between measurements on the same animal (six measurements, one for each brain region), mixed effects models were employed, which in essence is a MANOVA-type analysis (Goldstein 2003). If the 3-way interaction term (region $\times$ group $\times$ time) was not significant, it was omitted and the model was refitted. The non-significant 2-way interaction terms were similarly removed using one-by-one backward elimination. Inferences regarding the comparison between intruders, residents, and controls were based on the final models.

Immunoblot data was analyzed using a similar approach to the qPCR assessment. The immunoblot values were modeled as a function of region (a factor with two levels: hippocampus and midbrain), group (a factor with three levels: resident, intruder, and control) and their interaction. A significant interaction effect would indicate that the relationship between residents, intruders and control with respect to immunoreactivity depends on the specific brain region.

Significant omnibus tests for group effect (interaction between group and any of the other factors, or main effect of group), based on the models described above, were followed by pair-wise comparison between any two of the three groups. *P* values for these follow up pair-wise contrasts are reported unadjusted for multiple comparisons. Significance was judged at the level ($\alpha = 0.05$), two-sided. All analyses were performed using Proc Mixed in SAS[®] software (SAS Institute Inc 2009).

Results

Behavioral assessment

The application of the resident–intruder paradigm resulted in 33 intruders and 30 residents. The two groups differed on the behavioral rating, with the residents being significantly more aggressive ($M = 1.46$, $SD = 0.52$, range 1.00–2.79) than the submissive intruders ($M = 3.13$, $SD = 0.64$, range 1.79–4.00); $P < 0.001$.

Group effects on COMT and TH gene expression

In the model of COMT gene expression, the 3-way interaction terms region $\times$ time $\times$ group was not significant, and neither was the group $\times$ time interaction. The group $\times$ region (test on 10 df) and time $\times$ region (test on 5 df) terms were significant ($P < 0.001$). COMT expression depended on group, and this dependence was different within various regions of the brain, but the relationships between the groups did not depend on time. Compared to controls, resident mice had significant up regulation of COMT expression in the cerebellum ($P = 0.006$), frontal cortex ($P = 0.015$), hippocampus ($P < 0.001$), midbrain ($P < 0.001$), and striatum ($P = 0.025$). See Fig. 2 for the means and 95% confidence intervals (CIs) for the respective brain regions. In these five regions, COMT expression levels for controls was between those of residents and intruders, and the residents had significantly higher levels than the intruders (all P values < 0.001 , except for striatum, $P = 0.035$; Fig. 1). Additionally, intruders had significant down regulation compared to control mice selectively within the hippocampus ($P < 0.001$; Fig. 2). No difference between the groups was observed within the pons.

The final model for TH qPCR expression was similar to the COMT expression model, with no 3-way and no group $\times$ time interactions, and with significant group $\times$ region and time $\times$ region effects ($P < 0.001$). TH levels were highly expressed within the midbrain (as expected) and approximately fivefold higher than the relatively low expression in the pons (Fig. 3). TH expression within the cerebellum, frontal cortex, hippocampus, and striatum were at the limit of detection due to low abundance in these regions. TH levels in the midbrain were up regulated for residents and down regulated for intruders as compared to controls ($P < 0.001$; Fig. 3).

Effect of time from behavioral test to sacrifice of the animal

According to the statistical models, time did not affect the relationship between groups with respect to COMT and TH gene expression levels in the six brain regions. However, time had an effect on COMT and TH levels, and this effect was different at different regions. Specifically, COMT levels at 24 h were significantly higher than at 10 min in the cerebellum ($P < 0.001$), frontal cortex ($P < 0.001$), striatum ($P < 0.001$), and pons ($P = 0.013$), but were not different in the hippocampus and midbrain. Levels of TH at 24 h were lower than at 10 min for midbrain and pons ($P < 0.001$) and no different or below detection levels within the other regions.

Immunoblot analysis of COMT

Immunoblot analysis of COMT expression within the hippocampus and midbrain yielded two immunoreactive bands: a major band migrating at 28 kDa that corresponds to the membrane-bound form of COMT and a minor band migrating at approximately 24 kDa corresponding to the soluble form of COMT. The 28 kDa band was used for quantitative analysis, similar to independent reports of COMT expression in brain extracts (Chen et al. 2004). The 24 kDa band is expressed minimally in brain tissues, and was barely detectable in the hippocampal and midbrain homogenates. TUBB protein levels were not differentially

regulated in control, resident, or intruder subjects, and were used to normalize COMT expression levels.

COMT expression measured via immunoblot analysis showed differences between residents, intruders, and controls consistent with the qPCR assessments in the two areas where it was assessed: hippocampus and midbrain. In the hippocampus, compared with controls, COMT expression of residents was significantly up regulated ($P < 0.01$; Fig. 4), whereas COMT expression was down regulated within intruders ($P < 0.001$), and the difference between intruders and residents was significant ($P < 0.001$). In the midbrain, residents had significantly higher levels of COMT compared to controls and intruders ($P < 0.001$); the intruders had higher COMT levels than controls ($P = 0.014$; Fig. 4).

Discussion

Significant up regulation of COMT gene expression in residents was observed in the cerebellum, frontal cortex, hippocampus, midbrain, and striatum compared to intruders and control mice. Intruders also had significant down regulation of COMT gene expression within the hippocampus. Immunoblot analysis demonstrated COMT protein up regulation in the midbrain and hippocampus of residents and down regulation in intruders. Furthermore, TH qPCR analysis indicated significant up regulation in the midbrain of residents and down regulation in intruders. These findings underscore regional regulation of COMT and TH expression in aggressive and submissive behaviors. The resident–intruder paradigm is a suitable model to assess both aggressive behaviors as well as social defeat. Mechanisms underlying complex behaviors associated with psychopathology are challenging to study due to many factors including the involvement of multiple neural networks and the difficulty associated with generating appropriate animal models. Notwithstanding these roadblocks, relevance towards understanding the neurobiological basis of human psychiatric disorders demands the initiation of rational studies of several behaviors, including aggression. A multidisciplinary approach of behavioral characterization, molecular and cellular biology, immunoblotting, and functional genomics techniques are utilized as part of the experimental design of our program. Particular emphasis is placed upon analyzing relevant brain regions in vivo as a means of understanding cellular events occurring locally within specific regions following aggressive behaviors.

Aggressive mouse behavior differentially affected COMT regulation within distinct brain regions of residents and intruders. Pronounced up regulation of COMT PCR product expression was found in several discrete brain regions. Notably, up regulation of COMT expression within the midbrain of residents suggests this complex area as one of the sites important for the initiation of aggressive activity, particularly in light of the fact that mesolimbic and mesostriatal dopaminergic circuits originate here (with TH as the rate limiting enzyme in catecholaminergic biosynthesis) and project to areas such as the hippocampus and frontal cortex that also displayed significant up regulation of COMT expression. In contrast, significant down regulation of COMT qPCR product expression was observed in the hippocampus of intruder subjects, implicating a reduction of hippocampal COMT levels in social defeat. Accordingly, additional studies within independent models of aggression and social defeat are indicated, which may be useful to corroborate the present findings in the resident–intruder paradigm. Several lines of evidence suggest that the hippocampal–amygdaloid complex is involved in processing emotional experiences and related behaviors. Fear, reaction to unpleasant stimuli, and aggressive behavior are among the phenomena related to hippocampal functioning, and may be responsive to local COMT levels. The complex regulation of COMT expression within different brain regions underscore the importance of evaluating regional and cellular effects of aggressive behaviors throughout the central nervous system. Based on the present observations in COMT and TH

gene expression, additional functional genomics studies, including microarray analysis, are warranted at the cellular level within the midbrain (e.g., neurons within the substantia nigra and ventral tegmental area) and hippocampus (e.g., CA1 pyramidal neurons, CA3 pyramidal neurons, and dentate gyrus granule cells) and possibly within the frontal cortex, cerebellum, and striatum to characterize individual cell types directly linked to COMT regulation following the execution of aggressive and/or submissive behaviors.

COMT inactivates catecholamines through catalyzing the transfer of methyl groups from *S*-adenosylmethionine to the hydroxyl groups of catecholamines (Kopin 1994), and is a candidate gene for the control of aggressive behavior. Although COMT and TH are not likely to be the only candidate genes underlying aggressive behaviors, or even the only genes responsible for the catecholaminergic activation that is associated with aggression, they are indicated for assessment based on their differential regulation following the resident–intruder paradigm as well as other behavioral measurements of aggressive activity. Future microarray studies will help to identify other candidate genes, including those within catecholaminergic neurotransmission pathways as well as other novel candidate genes and signal transduction pathways. Importantly, catecholamine agonists increase aggressive behavior in animals and humans (Volavka et al. 2004; Volavka 2002; Miczek et al. 2002). Thus, low level of COMT activity would be expected to increase the likelihood of aggression. Accordingly, increased aggressive behavior was observed in COMT knockout mice (Gogos et al. 1998). Moreover, studies have reported an association between aggressive behavior in schizophrenics and low COMT activity (met allele), in North American (Volavka et al. 2004; Strous et al. 1997; Lachman et al. 1998), Israeli (Strous et al. 2003; Kotler et al. 1999), and Korean patients (Han et al. 2006), but not in large samples of British patients (Jones et al. 2001; Zammit et al. 2004). The met allele was associated with aggressive personality traits in a sample of German suicide attempters and controls (Rujescu et al. 2003), but not in Turkish patients with mental retardation (Isir et al. 2010). Thus, the preponderance of evidence seems to support some relationship between the COMT met allele and aggression in humans, but the evidence is largely based on samples of psychiatric patients, and is inconsistent even in such populations.

Although a decrement in COMT levels is predicted along with increased catecholaminergic activity in aggressive subjects, the observed increase in regional COMT levels in residents in the present report may be a neuroadaptive response to heightened and sustained dopaminergic tone elicited by the aggressive behavior. Indeed, significant elevation of dopamine was detected within the prefrontal cortex and nucleus accumbens in the first 10 min (that peaked 20–30 min after the confrontation) during a resident–intruder paradigm in rats (van Erp and Miczek 2000). The observed increase in dopamine levels was sustained for up to an hour after the resident–intruder interaction (van Erp and Miczek 2000). Conversely, social defeat in the intruder group may have an opposite effect on hippocampal catecholamine activity, with subsequent decrements in COMT levels as a neuroadaptive response over the trial period. A relationship between the gene dose (met allele) and hippocampal activation was demonstrated in humans (Smolka et al. 2005). Therefore, expression levels of COMT in the hippocampal formation (and possibly other regions receiving catecholaminergic afferent projections) vary continuously in response to environmental pressures and contribute to neuroadaptive behaviors in humans and animal models. Future experiments using fewer trial days for the test procedure may shed light on the neuroadaptive process(es) that may occur during the course of the resident–intruder paradigm. In addition, pharmacological challenge with amphetamines is hypothesized to exacerbate aggressive interactions, whereas antipsychotic medication delivery is expected to reduce aggressive behaviors in the resident–intruder paradigm, and may have pronounced effects of COMT regulation that merits investigation within this model system.

A discrepancy exists between the current findings and reports employing the resident–intruder paradigm utilizing a combined analysis of the midbrain and pons. Specifically, a decrease in COMT gene expression was found in subjects determined to be ‘winners’ (aggressive mice) versus ‘losers’ (submissive mice) at 20 days (but not 10 days) post test procedure (Kudryavtseva et al. 2004). Possible explanations for these discrepant findings include variable COMT levels in different mouse strains, as evidenced by a microarray analysis in mouse hippocampus evaluating eight strains of mice (Fernandes et al. 2004). Notably, the strongest genetic correlation between >200 probe sets in the eight inbred strains and a ranking of these strains by aggression was found for COMT (Fernandes et al. 2004). Other potential reasons for the divergent results include different qPCR-based strategies, and the use of a combined dissection that includes the pons and midbrain, whereas the present report evaluated the midbrain and pons separately, with significant results only observed in the former structure. The additional regions that were analyzed in the current report were not evaluated previously, precluding comparisons of COMT expression levels within the cerebellum, frontal cortex, hippocampus, or striatum. The aforementioned group has also reported an increase in TH and dopamine transporter mRNA levels within the ventral tegmental area of aggressive mice in the resident–intruder paradigm (Filipenko et al. 2001), which is consistent with our observation of differential TH regulation in the midbrain of residents and intruders.

Our long-term research endeavor is to identify signaling pathways and networks that regulate aggressive behavior through transcriptomic and proteomic approaches in relevant animal models. In the current study, the scope of work was more focused, as we tested whether the resident–intruder paradigm generated regionally-specific changes in expression for discrete catecholaminergic markers relevant towards current pharmacotherapeutic interventions employed to curtail excess aggressive behaviors. We identified relevant individual gene and encoded protein levels within specific regions that presumably regulate aggressive behavior, providing feasibility for embarking on large scale, expression profiling studies within this mouse model of aggression and social defeat. Ultimately, our goal is to identify targets for pharmacotherapeutic interventions to attenuate aberrant aggressive behaviors in humans with mental illness. Molecular and cellular paradigms include proteomic approaches, which have identified a few candidates, mainly abundant cytosolic proteins (Carboni et al. 2006). The heterogeneity of aggressive behavior and multiple neuronal systems involved is a challenge for developing rational treatments. The use of molecular and cellular approaches may enable the promise of future pharmacotherapeutic treatments of aggressive behavior developed on the basis of individual genotype-phenotype correlations including the assessment of the COMT gene and aggressive status.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Schematic diagram illustrating the micropunch dissection paradigm from the six selected regions in a rostrocaudal orientation. Representative coronal images of a C57BL/6 mouse brain atlas from a web based application (Williams et al. 1999) were adapted to illustrate approximate dissections for orientation and clarity of the experimental method. Frozen tissue from the micropunched regions (frontal cortex, striatum, hippocampus, midbrain, pons, and cerebellum) were placed in microfuge tubes until use in the qPCR assays

Fig. 2.

Means and 95% CIs for regional COMT qPCR product assessment in controls (*C*), intruders (*I*), and residents (*R*) using the ddCt method with GAPDH as a normalizing agent.

Significant up regulation of COMT expression was found in residents compared to controls and intruders within the cerebellum, frontal cortex, hippocampus, midbrain, and striatum. In addition, significant down regulation of COMT expression in the intruders was observed within the hippocampus

Fig. 3.

Means and 95% CIs for regional TH qPCR product assessment in controls (*C*), intruders (*I*), and residents (*R*). TH levels were highly expressed within the midbrain as compared to the pons, and at the limit of detection within the cerebellum, frontal cortex, hippocampus, and striatum. TH in the midbrain was up regulated in residents and down regulated in intruders, respectively, as compared to controls

Fig. 4. Means and 95% CIs for COMT immunoblot analysis in controls (*C*), intruders (*I*), and residents (*R*) within the hippocampus and midbrain (values normalized by TUBB expression). In the hippocampus, COMT expression of residents was significantly up regulated, whereas COMT expression was down regulated within intruders. In the midbrain, residents had significantly higher COMT levels compared to controls and intruders, and the intruders had slightly higher COMT levels than controls. The inset depicts a representative immunoblot demonstrating differential regulation of COMT (*upper band*) normalized to TUBB expression (*lower band*) within the hippocampus of control (*C*) and intruder (*I*) mice as compared to resident (*R*) mice