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Effects of immobilization stress on neurochemical markers in the motivational system of the male rat

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Abstract

Mesolimbic regions involved in motivated behavior are altered in animals undergoing repeated exposure to social stress. Here we test the hypothesis that other forms of persistent stress would also influence these same endpoints. Adult male Sprague-Dawley rats were exposed to immobilization stress either once (2h) or repeatedly (2h $\times$ 10d) and brains were harvested immediately after the last immobilization. A trio of indirect markers associated with dopaminergic activity was measured including dopamine transporter (DAT)- and dopamine D2 receptor subtype (D2r)-ligand levels as well as mRNA levels of the endogenous opioid enkephalin (ENK-mRNA). A single 2h session of immobilization stress produced an increase in striatal ENK-mRNA levels and DAT ligand binding compared with group-housed controls. In animals undergoing repeated immobilization stress and singly-housed post-stress, we found a significant reversal in direction of ENK-mRNA levels and DAT binding in the striatum, in addition to an increase in D2r binding density in the shell of the nucleus Accumbens compared with single-stress exposed rats. In another experiment using the same stress paradigm but allowing pair-housing post-stress, we found no alteration of ENK-mRNA but significant increases in DAT- and D2r-binding in the dorsal striatum. A major difference between single- and group-housing is the habituation of the corticosterone (CORT) stress response over 10d stress in group-housed rats. The present results parallel previous findings by our laboratory that repeated stress results in a relative reduction of ENK-mRNA levels and increased D2r-binding density in the striatum of rats. Furthermore, our data are consistent with the hypothesis that chronic stress induces an allostatic attenuation of the mesolimbic dopaminergic system in animals that do not habituate to the stressor, possibly due in part to persistent CORT elevations.

Keywords

Corticosterone; Dopamine; Enkephalin; Habituation; Opioid peptides; Stress

1. Introduction

Previously, we have reported that mesolimbic regions involved in motivated behavior show altered levels of indirect markers of dopamine activity several weeks after the termination of

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repeated exposure to social stress (Lucas et al., 2004). Specifically, we found decreased enkephalin-mRNA levels along with decreased dopamine transporter- and elevated dopamine D2 receptor-ligand binding levels in the nucleus Accumbens (Acb) in subordinate adult male rats. These results suggested a net blunting of dopamine tone in the motivational circuit of subordinate animals caused by repeated exposure to social stress.

However, it is generally accepted that dopamine release is increased in mesocorticolimbic regions after an acute stressor (Kalivas and Duffy 1995; Rouge-Pont, et al. 1998; Tidey and Miczek 1996). Indeed, social stressors in the form of the resident-intruder model have been reported to induce increases in dopamine release specifically in the Acb and prefrontal cortex but not in the lateral striatum (Tidey and Miczek 1996). Furthermore, previously socially-defeated rats display increased cocaine self-administration compared with non-defeated rats several days after repeated exposures to the stressor (Haney, et al. 1995; Tidey and Miczek 1997).

A possible explanation of these results could be that with repeated stress there is a net upregulation of dopamine receptors in response to a stress-induced blunting of dopamine. In fact, Gambarana and colleagues (Gambarana, et al. 1999) have shown that a relative decrease in dopamine release can occur after chronic stress. Thus, decreased dopamine tone could lead to an increased sensitivity to the psychostimulant effects of cocaine in repeatedly socially-defeated animals. But what could be causing the tonic decrease in accumbal dopamine?

A number of studies have provided evidence that stress can regulate the mesocorticolimbic enkephalinergic system (Angulo, et al. 1991; Bertrand, et al. 1997; Drolet, et al. 2001; Kalivas and Abhold 1987; Lucas, et al. 2004; Nikulina, et al. 2005; Stein, et al. 1992) and enkephalin (ENK) has been shown to positively affect the release of mesocorticolimbic dopamine (Kalivas and Abhold 1987). However, the long-term effects of stress on ENK or opioid receptors has only recently begun to be investigated (Lucas, et al. 2004; Nikulina, et al. 2005).

Interestingly, enkephalinergic neurons in the striatum selectively express dopamine D2 receptors (Curran and Watson 1995; Le Moine and Bloch 1995; Maldonado, et al. 1990) although small clusters of cells in the rostral pole and shell of the nucleus Accumbens do not (Curran and Watson 1995). Some studies have indicated that dopamine D2 receptors (D2r) are altered after social stress where social subordination in cynomolgus female monkeys results in decreased D2r function (Shively 1998) or no change in receptor number (Morgan, et al. 2002) but increased vulnerability to cocaine self-administration. Finally, several laboratories have reported a decrease in dopamine transporter (DAT) binding in animals that have experienced social stress (Isovich, et al. 2001; Isovich, et al. 2000; Rodriguiz, et al. 2004).

Therefore, the goal of the present study was three-fold: 1) to determine if other forms of stress other than social stress can also induce changes in DAT, D2r, and ENK-mRNA levels in the Acb and associated striatal brain regions; 2) to provide evidence that long-lasting alterations in these mesolimbic markers appear during the course of the repeated stress; and 3) to assess whether habituation to repeated stress and loss of hypothalamic-pituitary-adrenal axis reactivity can be correlated to these markers.

2. Results

2.1 Serum corticosterone response to acute and repeated stress

Compared with pair-housed control animals, rats undergoing a single 2 h exposure to immobilization stress displayed a 3-fold increase in serum corticosterone (CORT) levels ($F_{1,7} = 5.64$, $p = 0.049$, Fig. 1A). Single-housed rats experiencing periodic/chronic immobilization stress ($10 \text{ d} \times 2 \text{ h/d}$) also showed higher serum CORT levels than single-housed control animals

($F_{1,7} = 21.3$, $p = 0.003$, Fig. 1B). However, pair-housed rats undergoing the identical periodic/chronic immobilization stress as the previous group did not evidence any significant difference in CORT levels compared with pair-housed controls ($F_{1,7} = 0.10$, $p = 0.92$, Fig. 1C). In addition, a comparison of control CORT levels indicated that they were higher for both single- and pair-housed chronic stress groups in contrast to single-immobilization controls ($F_{2,21} = 4.13$, $p = 0.03$).

2.2 Enkephalin-mRNA levels

A significant main effect of stress treatment was found in rats undergoing a single 2 h immobilization ($F_{1,13} = 5.07$, $p = 0.042$) resulting in higher enkephalin-mRNA in stressed vs. control rats (Fig. 2A). The interaction term between Treatment and Striatal subregions was not significant. In rats that were single-housed and received periodic/chronic stress and which did continue to show hypothalamic-pituitary-adrenal axis (HPA) responses to stress, there was a significant main effect due to stress ($F_{1,14} = 12.1$, $p = 0.004$) but no significant interaction between Treatment and Striatal subregion. However, the direction of change was in the opposite direction from that observed in rats undergoing acute stress: i.e., significant decreases in enkephalin-mRNA levels were seen in the striatum compared with single-housed controls (Fig. 2B). Interestingly, pair-housed rats experiencing periodic/chronic stress, and which showed habituation of the HPA response during repeated stress, demonstrated no differences from controls in any of the striatal subregions examined ($F_{1,14} = 1.58$, $p = 0.23$, Fig. 2C).

2.3 Dopamine D2 receptor ligand binding levels

Comparing dopamine D2 receptor ligand binding levels between pair-housed controls and rats undergoing a single-exposure to immobilization stress yielded no significant differences in main effects due to stress or among striatal subregions ($F_{1,7} = 2.99$, $p = 0.12$, Fig. 3A). Single-housed animals undergoing periodic/chronic immobilization did exhibit a significant interaction between stress treatment and striatal subregions ($F_{3,21} = 3.78$, $p = 0.026$) in that only the shell of the nucleus Accumbens subregion demonstrated an increase in dopamine D2 receptor ligand binding levels compared with single-housed controls (Fig. 3B). Pair-housed rats experiencing periodic/chronic immobilization demonstrated a significant interaction term as well between stress treatment and striatal subregion ($F_{3,21} = 3.78$, $p = 0.025$, Fig. 3C) with dorsolateral caudate putamen subregion indicating increased binding levels compared with pair-housed controls. Thus there was no acute stress effect on D2 receptor ligand binding but there was a modest elevation by chronic, non-HPA habituating stress of D2 binding in AcbSh that was absent in group housed rats given chronic stress where HPA habituation occurred.

2.4 Dopamine transporter ligand binding levels

There was a significant main effect of stress treatment of rats undergoing single exposure to immobilization stress ($F_{1,7} = 5.07$, $p = 0.059$) with no significant interaction between Treatment and Striatal subregions resulting in higher dopamine transporter-binding in stressed vs. control rats (Fig. 4A). Periodic/chronic stress in non-HPA habituating single-housed rats did result in a significant main effect due to stress ($F_{1,6} = 9.48$, $p = 0.021$; Fig. 4B) but no significant interaction term. Here, a reversal in direction (i.e., decreased levels compared with controls) from that observed with single exposure to immobilization stress was observed in the striatum (Fig. 4B). On the other hand, HPA habituating pair-housed rats undergoing periodic/chronic stress displayed a significant main effect of stress treatment ($F_{1,7} = 8.24$, $p = 0.023$) and interaction term ($F_{3,21} = 3.20$, $p = 0.04$, Fig. 4C), whereby ligand-binding levels in dorsolateral and dorsomedial caudate putamen subregions were increased compared with pair-housed controls. Thus, chronic stress where HPA responses did not habituate produced opposite effects to chronic stress where HPA responses did habituate. Moreover, chronic non-habituating stress produced an opposite effect to that of acute stress.

3. Discussion

We have previously investigated whether repeated stress has long-lasting effects on mesolimbic markers of dopaminergic activity by using a rodent model of social stress (Lucas, et al. 2004). In the current study, our goal was to determine if other forms of long-term stress resulted in similar activation of these regions and to compare acute and chronic stress effects, including a form of chronic stress in which the hypothalamic-pituitary-adrenal (HPA) response habituates. Compared with controls, elevated stress hormone levels at the time of sacrifice coincided with increased enkephalin-mRNA and dopamine transporter levels in striatal subregions in rats undergoing single exposure to immobilization stress. However, after repeated immobilizations over the course of several days in rats that were singly-housed and which showed no habituation of the HPA response to stress, enkephalin-mRNA and dopamine transporter levels showed a decrease whereas dopamine D2 receptor binding levels increased compared with singly-housed controls. In contrast, rats that were pair-housed with cage-mates after repeated immobilization sessions displayed habituation and had stress hormone levels that were similar to pair-housed controls. In these animals, enkephalin-mRNA levels were similar between control and repeated-stress conditions, whereas, both dopamine D2 receptor- and dopamine transporter-ligand binding levels were elevated in dorsal striatal subregions.

The secretion of corticosterone (CORT) is one of the most significant aspects of the stress response. In the present report, the presence or absence of a habituation of CORT secretion to repeated stress appears to be associated with the different responses observed in several indirect markers of dopaminergic activity. The fact that control levels of CORT appear elevated in rats undergoing chronic stress compared with those in the single stress experiment could be due to chronic stress animals being housed in the same room after the daily immobilization session. Thus, controls in the chronic treatment groups experience a phenomenon previously identified as an "...intraspecific communication of the intensity of stress" (Pitman, et al. 1988) which presumably increases their CORT levels. Although CORT levels in chronic stress controls are elevated to acute stress levels, relative differences between single-housed and pair-housed chronic stressed groups exist in the present findings. It is the supra-acute CORT levels in the single-housed chronic stress group that we have identified as displaying a non-habituated stress response to periodic immobilizations. While the relatively elevated CORT response (compared with controls) in the acute stress and chronic single-housed stress groups indicates that rats are stressed at the time of sacrifice, the quantity of circulating CORT is more than twice as high in the chronic stress group. Therefore it is incorrect to assume that the CORT response is the same in both groups since the difference in magnitude of the CORT response, along with the length of the exposure to the stressor would suggest that the rat is experiencing stress phenomena that are at face value quite different.

Insight into the possible role of CORT on indirect markers of dopaminergic activity can be gleaned from studies of the effects of glucocorticoids on preproenkephalin-mRNA and enkephalin expression in a number of neural systems. For example, not only has the glucocorticoid, dexamethasone been reported to increase preproenkephalin-mRNA abundance in NG108-15 neuroblastoma-glioma hybrid cells but also has stimulation of adenylate cyclase activity through forskolin treatment led to parallel increases (Yoshikawa and Sabol 1986). Indeed, because rats were euthanized right after the stress session, the presence or absence of a CORT response to that stress may contribute to the observed outcome. Presence of an elevated or non-habituated CORT response in singly-housed, chronically stressed rats may drive the opposite observed effect on ENK-mRNA levels.

However, among the handful of studies that have examined the relationship between stress and enkephalin (Angulo, et al. 1991; Bertrand, et al. 1997; Drolet, et al. 2001; Kalivas and Abhold 1987; Lucas, et al. 2004; Nankova, et al. 1996; Stein, et al. 1992), the consensus appears to be

that stress alters extracellular levels of enkephalin in mesolimbic structures and that temporal aspects of stress determine either increases or decreases in enkephalin release or expression. For example, short term exposure to stress appears to result both in an increase in enkephalin release (Kalivas and Abhold 1987) and mRNA levels (Lucas, et al. 2004). On the other hand, chronic stress exposure is associated with decreased extracellular levels of enkephalin release as well as decreased enkephalin-mRNA abundance in striatal structures (Angulo, et al. 1991; Bertrand, et al. 1997; Lucas, et al. 2004). In fact, other investigators have reported that periodic/chronic stress results in a decreased number of ENK-mRNA positive cells in the ventral medulla of adult male rats (Mansi, et al. 2000).

Regarding stress effects on D2 receptors and dopamine transporters, a single exposure to social defeat does not result in a change in striatal dopamine transporter binding levels 4 hours after the stressor (Isovich, et al. 2001). That our results show an increase in dopamine transporter binding is not contradicted by these findings since we euthanized rats right after stress and dopamine transporter levels have been reported to be positively regulated by circulating stress hormone titers (Sarnyai, et al. 1998) which is consistent with elevated CORT levels at the time of sacrifice. In the present study, DAT binding levels are different in habituated vs. non-habituated chronic stressed groups. The shift in DAT binding to the dorsal striatum may suggest a role for the dorsal striatum in habituated chronic stressed rats.

Indeed, dopamine transporter levels may be on a downward trend in the study by Isovich and colleagues given that 24 hours later, binding levels reach a nadir, since a number of studies investigating the effects of repeated social stress suggest that dopamine transporter binding sites are downregulated immediately after the stressor (Isovich, et al. 2000) or for some time after the stressor has ceased (Lucas, et al. 2004; Meyer, et al. 2001). Another more recent study has reported that dopamine transporter uptake is increased after 7 days of daily 2-hour restraint stress in mice treated with the glucocorticoid receptor antagonist mifepristone (Copeland, et al. 2005). Although this study more closely parallels our experimental design of 11 daily sessions of 2-hour immobilization stress, it is difficult to reconcile differences in outcomes since our rats were sacrificed immediately after their last stress session and did not undergo antagonist treatment.

Chronic stress has been reported to decrease dopamine D2 receptor mRNA levels in the midbrain (Dziedzicka-Wasylewska, et al. 1997). To our knowledge, the present study is the first to investigate dopamine D2 receptor binding levels immediately after stress. Other groups have reported decreases in dopamine D2 receptor binding in the limbic forebrain but no changes in the striatum after chronic stress (Papp, et al. 1994). Consistent with our previous study using a social stress paradigm where we reported increased dopamine D2 receptor binding 3 weeks after the last social stress session (Lucas, et al. 2004), our present findings show increased binding levels immediately after the last session of repeated stress.

We have interpreted this changed landscape as caused by repeated stress to mean that there is an allostatic state (Koob and Le Moal 2001; McEwen 2000) in mesolimbic dopaminergic function where there is a generalized blunting of dopamine release. Evidence supporting this hypothesis can be seen in a recent study by Nikulina and colleagues (Nikulina, et al. 2005) that show not only an increased expression of mu-opioid receptor in the ventral tegmental area 7 days after repeated defeat stress (suggesting a compensatory adaptation to decreased enkephalin levels) but also an increased locomotor activation after intra-VTA infusion with the opioid agonist DAMGO. Indeed, Willner and colleagues (Willner, et al. 1992) have termed this phenomenon 'stress-induced anhedonia'.

In conclusion, a single exposure to immobilization stress resulted in increased enkephalin-mRNA and dopamine transporter ligand binding in the striatum of male adult rats that were

sacrificed immediately after the termination of the stress session. Animals undergoing repeated exposure to immobilization stress, in which the HPA response did not habituate because they were singly housed, demonstrated a reversal in direction of the two previously mentioned markers of mesolimbic dopaminergic activity in addition to displaying an increase in dopamine D2 receptor binding in the shell of the Acb immediately after the last stress session. Because the effects of repeated stress are not found in pair-housed rats for which HPA responses habituate, a role of CORT secretion is suspected and a reversal of its effects is suggested. We propose that in non-habituating conditions the dopaminergic system may become blunted via decreased enkephalin synthesis and release (Kalivas and Abhold 1987; Kelley, et al. 1980). A compensatory downshift in dopamine transporter number and increase in dopamine D2 receptors suggest an allostatic state of the dopaminergic system that in turn results in a hypersensitized response to psychostimulants in chronically stressed individuals.

4. Experimental Procedure

4.1 Subjects and treatments

Adult male Sprague-Dawley rats (Charles River, Kingston, NY), 250 to 300 g were kept under standard light/dark cycles (0700h lights on, 1900h lights off), *ad libitum* water and food conditions, and housed in plastic cages with bedding under the auspices of the accredited (American Association of Accreditation of Laboratory Animal Care) Rockefeller University Laboratory Animal Research Center. Upon arrival, rats were allowed to acclimate to their new environment for 1 week. After this, rats were either immobilized by placement into immobilization bags for 2 h (10:00 to 12:00) either once (Acute Immob Stress: n=8 and naïve Controls n=8) or once a day for 10 days (Chronic Immob Stress). Two separate experiments were tried with the Chronic Immob rats. In the first experiment rats were separated from their cage-mates and kept separate throughout the 10 days (n=8). Controls in this group were also separated from their cage-mates and kept in separate cages throughout the length of the 10 day period but were handled daily (n=8). In the second experiment, rats were pair-housed not only before the immobilization period but also afterwards with their cage-mates who had also undergone immobilization (n=8). Controls were left alone with their cage-mates throughout the length of the 10 days (n=8). Rats sacrificed immediately after the final immobilization period by decapitation and brains were quickly removed. Brains were fresh frozen, and stored at -70°C until sectioned at $20\mu\text{m}$ thickness and thaw-mounted onto slides. The slides were kept at -70°C until molecular analyses. Trunk blood was collected at time of sacrifice and plasma levels of corticosterone (CORT) were measured by radioimmunoassay kit (DPC).

4.2 In situ hybridization

For detection of enkephalin-mRNA A 970 bp fragment of rat preproenkephalin (PPE) cDNA in the pSP64 transcription vector was provided by Dr. Steven Sabol (NIH) and was linearized with *SacI* for antisense. Standard pre-hybridization, hybridization, and post-hybridization procedures were followed as previously published (Lucas, et al. 2004). Slides were apposed to Kodak X-OMAT X-ray film and exposed for 7 days at RT.

4.3 Dopamine transporter autoradiography

Slides were removed from -70°C freezer storage and brain sections were allowed to thaw for 10 min. at room temperature. Control and stress treatment sections were randomly selected and matched during processing to reduce between-slide variability. To determine specific dopamine transporter binding, sections were incubated in Buffer A: 137 mM NaCl, 2.8 mM KCl, 10 mM Na_2HPO_4 , 10 mM KH_2PO_4 , pH to 7.4 and finally adding 10 mM NaI plus 1.5 nM [^{125}I]RTI-121 (PerkinElmer), for 60 min. at room temperature. After this time, sections were washed twice for 20 min. each time with Buffer A at 4°C . Salts were removed from

sections with a quick rinse in ice-cold distilled water and slides were dried under forced air. Slides were apposed on Kodak X-OMAT film and exposed for 2 h.

4.4 Dopamine D2 receptor binding

Slide mounted sections were air dried for 3 min. after removal from -70°C storage; pre-incubated for 15 min. in 50mM Tris HCl (pH 7.4), 120 mM NaCl at room temperature; followed by an incubation for 30 min at room temperature in 50mM Tris HCl (pH 7.4), 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂ and a 0.6 nM concentration of [¹²⁵I]-iodosulpride. Slides were rinsed 2 times for 5 min each time in ice-cold 50mM Tris-HCl (pH 7.4) buffer and placed before a stream of room temperature air to dry for several hours. Once dried, slides were put in cassettes and exposed to Kodak X-OMAT film for 1 day.

4.5 Macroscopic semiquantitative image analysis

In situ hybridization, dopamine receptor, and dopamine transporter ligand binding X-ray film results were examined with a desktop illuminator (Kaiser Fototechnik, Buchen, Germany) and a CCD video camera (Hamamatsu C8484) with a Micro Nikkor lens (Nikon) attached. Microscale ¹⁴C standards for *in situ* hybridization were exposed on Kodak XAR film for 7 days and ¹²⁵I microscale standards (GE Healthcare Biosciences, Piscataway, NJ) for dopamine transporter and dopamine D2 receptor autoradiography were exposed on Kodak XAR film for 2 h and 1 d, respectively. Following this, autoradiographs were digitized using computer-assisted densitometry (Compix Imaging Systems, Sewickley, PA.). Background illumination was digitally subtracted and gray level/optical density calibration was done using the appropriate microscale standard for optical density. Optical density was plotted as a function of microscale calibration values. Five regions per half-section were selected for optical density quantitation in the region of the striatum: dorsolateral and dorsomedial caudate-putamen and core and shell of the nucleus accumbens plus a background reading taken from the corpus callosum. Four sections were analyzed in each hemisphere per rat and a mean optical density value was registered for each region in the striatum (resulting in a minimum of 8 data points per region of interest) resulting in one value for each region per animal. Background optical density values were subtracted from striatal regions of interest.

4.6 Statistical analysis

Serum corticosterone levels (µg/dL) were analyzed by a one-way analysis of variance (ANOVA) with Treatment (Control, Stress) as a between subject factor. Calibrated optical density measurements for *in situ* hybridization (nCi/g) was analyzed by a 2-way ANOVA with Treatment (Control, Stress) as a between subject factor and Striatal subregion (dorsolateral caudate-putamen, DLCPu; dorsomedial caudate-putamen, DMCPu; core, AcbC and shell of the nucleus accumbens, AcbSh) as a within-subject factor. Calibrated optical density measurements for dopamine transporter (RTI-121) and dopamine receptor ligand binding (nCi/mg) were analyzed by a 2-way ANOVA with Treatment (Control, Stress) and Striatal Region (dorsolateral and dorsomedial caudate-putamen; core and shell of the nucleus accumbens) as a within-subject factors since brain sections were processed in treatment pairs *a priori*. Post-hoc follow-up tests of main effects (Student-Newman-Keuls) and interaction effects were done as required with a two-tailed cut-off at $p < 0.05$ for significance. Data are represented as mean values $\pm$ SEM (standard error of the mean).

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Abbreviations

AcbC	core of the nucleus accumbens
AcbSh	shell of the nucleus accumbens
CON	control
CORT	serum corticosterone
DAT	dopamine transporter
D2r	dopamine D2 receptor
DLCPu	dorsolateral caudate putamen
DMCPu	dorsomedial caudate putamen
ENK	enkephalin
HPA	hypothalamic-pituitary-adrenal
Acb	nucleus Accumbens

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Fig. 1.

Serum corticosterone levels of trunk blood collected immediately after the end of immobilization stress. Fig. 1A shows a significant increase in corticosterone levels in stressed rats (Stress) compared with unstressed controls (Control) undergoing a single (Acute) 2 h immobilization. Fig. 1B displays a significant increase in corticosterone levels in single-housed stressed rats undergoing repeated (Chronic: 2 h/d $\times$ 10 d) immobilizations compared with single-housed controls. Fig. 1C shows no differences in corticosterone levels in pair-housed stressed rats undergoing repeated (Chronic: 2 h/d $\times$ 10 d) immobilizations compared with pair-housed controls. * $p < 0.05$, ** $p < 0.01$.

Fig. 2.

Enkephalin (ENK)-mRNA levels in the striatum immediately after the termination of immobilization stress. Fig 2A shows a significant increase in ENK-mRNA levels in stressed rats undergoing a single 2 h immobilization (Acute Immob) compared with unstressed controls (Control). Fig. 2B displays a significant decrease in ENK-mRNA levels in striatum of single-housed stressed rats undergoing repeated (Chronic: 2 h/d $\times$ 10 d) immobilizations (Chronic Immob) compared with single-housed controls (Control). Fig. 2C displays no differences between in ENK-mRNA levels in the striatum of pair-housed stressed rats undergoing repeated (Chronic: 2 h/d $\times$ 10 d) immobilizations (Chronic Immob) compared with pair-housed controls (Control). No significant interactions were found between Treatment and Striatal subregion. * $p < 0.05$.

Fig. 3.

Dopamine D2 receptor (Iodosulpride) ligand binding (D2r) levels in the striatum or several striatal subregions immediately after the termination of immobilization stress. Fig. 3A shows no significant differences in D2r binding levels in the striatum of stressed rats undergoing a single 2 h immobilization (Acute Immob) compared with unstressed controls (Control). No significant interaction between Treatment and Striatal subregion was found. Because of a significant interaction term, Fig. 3B displays a significant increase in D2r binding levels in the shell of the nucleus Accumbens in single-housed stressed rats undergoing repeated (Chronic: 2 h/d $\times$ 10 d) immobilizations (Chronic Immob) compared with single-housed controls (Control). In another significant interaction between Treatment and Striatal subregion, Fig. 3C shows a significant difference in D2r binding levels in DLCPu subregions in pair-housed stressed rats undergoing repeated (Chronic: 2 h/d $\times$ 10 d) immobilizations (Chronic Immob) compared with pair-housed controls (Control). Abbreviations: DLCPu, dorsolateral caudate-putamen; DMCPu, dorsomedial caudate-putamen; AcbC, core of the nucleus Accumbens; AcbSh, shell of the nucleus Accumbens. * $p < 0.05$.

Fig. 4.

Dopamine transporter (RTI-121) ligand binding (DAT) levels in the striatum or in several striatal subregions immediately after the termination of immobilization stress. No significant interaction between Treatment and Striatal subregion was found in Figs. 4A and 4B. Fig. 4A shows a significant increase in DAT binding levels in the striatum of stressed rats undergoing a single 2 h immobilization (Acute Immob) compared with unstressed controls (Control). Fig. 4B displays a significant decrease in DAT binding levels in the striatum of single-housed rats undergoing repeated (Chronic: 2 h/d $\times$ 10 d) immobilizations (Chronic Immob) compared with single-housed controls (Control). Because of a significant interaction term, Fig. 4C displays a significant increase in DAT binding levels in the dorsolateral and dorsomedial caudate putamen in pair-housed rats undergoing repeated (Chronic: 2 h/d $\times$ 10 d) immobilizations (Chronic Immob) compared with pair-housed controls (Control). Abbreviations are the same as found in Fig 3. * $p < 0.05$, ** $p < 0.01$.