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Neurochemical analysis of rat strain differences in the startle gating-disruptive effects of dopamine agonists

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Abstract

The disruption of prepulse inhibition (PPI) in rats by dopamine (DA) agonists is used to study the neural basis of strain differences in dopaminergic function. We reported that, compared to Long–Evans (LEH) rats, Sprague–Dawley (SDH) rats are more sensitive to the PPI-disruptive effects of the direct D1/D2 agonist apomorphine (APO) and the indirect DA agonist d-amphetamine (AMPH). This strain difference is heritable, with PPI drug sensitivity following a generational pattern (SDH>N2>F1>LEH) suggestive of additive effects of multiple genes. Here, we assessed the neurochemical bases for these heritable strain differences by measuring tissue levels of dopamine, serotonin (5HT) and their respective metabolites in several forebrain regions after vehicle, APO or AMPH administration. SDH rats were more sensitive than LEH rats to the PPI-disruptive effects of both APO (0.5 mg/kg) and AMPH (4.5 mg/kg). Several significant SDH vs. LEH strain differences in regional neurochemical levels were detected, as were drug effects on these chemicals. However, SDH, LEH and F1 rats did not exhibit differential drug sensitivity in any neurochemical indices measures. These findings suggest that inherited differences in the dopaminergic regulation of sensorimotor gating do not likely reflect differences in presynaptic forebrain dopaminergic or serotonergic processes.

Keywords: Amphetamine; Apomorphine; Dopamine; Medial prefrontal cortex; Nucleus accumbens; Prepulse inhibition; Schizophrenia; Startle; Striatum

1. Introduction

"Prepulse inhibition" (PPI) is the inhibition of startle when a weak, non-startling stimulus precedes a startling stimulus. PPI is an operational measure of sensorimotor gating (Graham, 1975): the weak "prepulse" is thought to trigger inhibitory processes that—for a very brief temporal window (e.g. 30–300 ms)—blunt the responsivity to subsequent stimuli, and thereby "protect" the information contained within the prepulse (Swerdlow, 1996). Many studies have demonstrated that PPI is impaired in several human neuropsychiatric disorders, particularly schizophrenia (Braff et al., 1978; Grillon et al., 1992; Bolino et al., 1994; Schall et al., 1996; Karper et al., 1996; Kumari et al., 1999; Weike et al., 2000; Parwani et al., 2000; Ludewig and Vollenweider, 2002; Mackeprang et al., 2002; cf. Braff et al., 2001). In rats, PPI is reduced by dopamine (DA) agonists such as the DA releaser d-amphetamine (AMPH) and the mixed D1/D2 agonist apomorphine (APO) (Swerdlow et al., 1986; cf. Geyer et al., 2001); the ability of drugs to restore PPI in APO-treated rats predicts antipsychotic potency (Swerdlow et al., 1994).

Strain differences in the PPI-disruptive effects of DA agonists have been identified in outbred and inbred rat populations (Rigdon, 1990; Swerdlow et al., 1997, 2000a,b, 2001a,b, 2004a,b). For example, Harlan Sprague–Dawley (SDH) rats are more sensitive than Harlan Long–Evans (LEH) rats to the PPI-disruptive effects of APO (Swerdlow et al., 2001a,b). This strain difference is neurochemically specific to DA agonists (i.e. is not seen with NMDA antagonists or 5HT agonists; Swerdlow et al., 2004a), it is stable across breeding and testing facilities (Swerdlow et al.,

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2001b), is evident on or before day 18 of life and after central as well as peripheral administration of APO (Swerdlow et al., 2002). Most germane to the present line of inquiry, these SDH vs. LEH strain differences are inherited with a pattern that suggests simple additive effects of multiple genes (Swerdlow et al., 2004b). Our group has sought to understand the neural basis of this inherited sensitivity to a DA-mediated loss of sensorimotor gating, which might serve as a model for the loss of PPI associated with inherited perturbations of DA function commonly seen in outbred human populations (e.g. schizophrenia and Tourette Syndrome) (cf. Braff et al., 2001).

It is possible that the neural basis for this heritable difference in PPI drug sensitivity reflects differential drug effects on DA, its release or metabolism within forebrain regions that regulate PPI in SDH and LEH rats. In order to test this possibility, we assessed regional forebrain neurochemical measures in SDH, LEH and F1 (SDH×LEH) rats in response to the DA agonists APO and AMPH, over the time course (10–60 min post-administration) during which differences in PPI sensitivity are observed. Because DA is the common neurochemical substrate implicated in the disruption of PPI by both AMPH and APO, measures primarily assessed indices of DA content, release and metabolism (3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (MeTYR)). Measures of serotonin (5HT) and its major metabolite (5HIAA) were also performed based on evidence that AMPH stimulates 5HT release (Kuczenski and Segal, 1989; Rothman et al., 2001), that SD vs. LE strain differences in stimulant effects have been attributed by some to reflect differences in serotonergic mechanisms (Horowitz et al., 1997), and that PPI is also regulated by serotonergic mechanisms (cf. Geyer et al., 2001). Parallel behavioral studies were also conducted in SDH and LEH rats.

2. Methods and materials

2.1. Experimental animals

A total of 180 adult rats were used in these experiments. Rats were involved in two different studies: (1) a within-subject study of the effects of APO (0.5 mg/kg sc), AMPH (4.5 mg/kg sc) or saline vehicle on PPI in SDH (n=12) and LEH (n=12) rats; (2) a between-subject study of the neurochemical effects of these treatments in SDH (n=48), LEH (n=48) and F1 (SDH×LEH) rats (total n=60; male n=35, female n=25). SDH and LEH rats were obtained as adults from commercial suppliers (Harlan Laboratories; SDH: San Diego, CA; LEH: Indianapolis, IN). Methods for housing and all behavioral testing were consistent with the substantial literature of startle measures in rodents (cf. Geyer and Swerdlow, 1998). To produce an F1 (SDH×LEH) generation, SDH and LEH rats were reciprocally crossed (with representation of both sexes from both strains). Pregnant female LEH and SDH rats were housed individually. F1 litters were allowed to mature to adulthood without drug testing, and were handled regularly beginning at day 50. Aside from the strain of the nursing female rat, rearing conditions for all F1 pups were comparable. Adult F1 male and female rats were housed in same-sex rooms in groups of two to four.

After shipment arrival, SDH and LEH rats obtained from commercial vendors were handled within 48 h, and were maintained in the housing facility for at least 1 week prior to behavioral testing. A reversed 12 h light/dark cycle was used (lights on at 19:00 h, off at 07:00 h) for at least 1 week prior to testing. All testing and drug administration occurred between 10:00 and 17:00 h. Rats were handled regularly prior to any procedures to minimize stress during behavioral testing, and were given ad libitum access to food and water except during behavioral testing. Throughout these studies, all efforts were made to minimize animal suffering and to reduce the number of animals used. All experiments conform to guidelines of the National Institute of Health for the use of animals in biomedical research and were approved by the Animal Subjects Committee at the University of California, San Diego (protocol #S01221).

2.2. Drugs

APO (0.5 mg/kg) was administered to rats in a saline/0.1% ascorbate vehicle. The 0.5 mg/kg dose of APO yields significant PPI differences but comparable drug levels in SDH and LEH rats within forebrain regions that regulate PPI (Swerdlow et al., 2002). AMPH (4.5 mg/kg) was administered to rats in a saline vehicle. The 4.5 mg/kg dose of AMPH yields significant PPI differences in SDH and LEH rats (Swerdlow et al., 2003). A “vehicle” treatment of physiological saline was used as a comparison dose for both APO and AMPH. Treatments were administered subcutaneously (sc) to rats 5 min prior to behavioral testing, or 10, 20, 30 or 60 min prior to sacrifice for HPLC measures.

2.3. Apparatus

Startle experiments used four startle chambers (SR-LAB; San Diego Instruments, San Diego, CA) housed in a sound-attenuated room with a 60 dB ambient noise level. Each startle chamber consisted of a Plexiglas cylinder (8.7 cm internal diameter) resting on a 12.5×25.5 cm Plexiglas stand. Acoustic stimuli and background noise were presented via a Radioshack Supertweeter mounted 24 cm above the Plexiglas cylinder. Startle magnitude was detected and recorded as transduced cylinder movement via a piezoelectric device mounted below the Plexiglas stand. Response sensitivities were calibrated (SR-LAB Startle Calibration System) to be nearly identical in each of the two startle chambers (maximum variability <1% of stimulus range and <5% of response ranges). Chambers were also balanced across all experimental groups. Sound levels were

measured and calibrated with a sound level meter (Quest electronics: Oconomowoc, WI), A scale (relative to 20 μN/M2), with microphone placed inside the Plexiglas cylinder. Methodological details can be found in published material (Geyer and Swerdlow, 1998).

2.4. Startle testing procedures

Approximately 7 days after shipment arrival, rats were exposed to a brief "Matching" startle session, as described previously (Swerdlow et al., 2002). Rats were placed in a startle chamber, and exposed to 5 min of 70 dB background noise followed by 17 PULSE trials of 40 ms 120 dB noise bursts ("PULSE") and 3 PREPULSE+PULSE trials consisting of a 20 ms 82 dB (12 dB above background) prepulse followed 100 ms by a 120 dB pulse (onset to onset). Data from this session were used to assign rats to balanced dose groups, according to their average level of PPI.

Behavioral testing continued 2–4 days after the "Matching" session. SDH and LEH rats were brought to the laboratory in individual cages, >1 h before testing. APO (0.5 mg/kg sc), AMPH (4.5 mg/kg sc) or saline was administered, and 5 min later rats were placed into the startle test chambers. Test sessions were approximately 19 min long and consisted of 5 min of 70 dB background followed by five trial types: PULSE, prepulse trials (20 ms noise burst 5, 10, or 15 dB above background followed 100 ms by PULSE) and NOSTIM trial. The session consisted of four "blocks": blocks 1 and 4 consisted of pulse alone trials (4 initial and 3 final), separated by blocks 2 and 3 which included 10 repetitions of each prepulse trial (5 trials each per block) and 16 pulse trials (8 trials per block) in pseudorandom order. Intertrial intervals were variable and averaged 15 s. In addition, "NO STIM" trials were placed between each stimulus trial (measuring cage activity without stimulus delivery) to assess gross motor activity during the test session, but were not included in the calculation of intertrial interval. The test was repeated three times, with 4–5 days between tests; rats received a different drug prior to each test. In this manner, treatment (saline, APO or AMPH) was a between-subject variable. Drug dose order was balanced across drugs and rat strains.

2.5. Neurochemistry

SDH, LEH and F1 rats were treated with saline, APO or AMPH, as above. Decapitation 10, 20, 30 or 60 min later was followed immediately by brain removal and cooling on a steel plate immersed in ice. The medial prefrontal cortex (mPFC), nucleus accumbens (NAC), anterior striatum (AMS) and posterior striatum (PS) were removed by free-hand dissection from wire-guided coronal sections as described previously (Swerdlow et al., 1986). Tissue was frozen on dry ice and stored at −40 °C until assayed for neurochemical levels using high performance liquid chromatography (HPLC). Regional brain levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (MeTYR), 5-hydroxyindoleacetic acid (5HIAA), and serotonin (5HT) were assessed using methods modified from Schmidt et al. (1990). Briefly, tissue samples were sonicated in 0.5 ml ice-cold 0.1 N perchloric acid, then centrifuged for 15 min at 10,000 rpm, and an aliquot of the supernatant was assayed using HPLC with electrochemical detection as previously described (Kuczenski et al., 1995). The HPLC-EC consisted of a 100×4.6 mm ODS-C18 3 column (Regis) maintained at 40 °C. Mobile phase (0.05 M citric acid, 7% methanol, 0.1 mM Na_2EDTA and 0.2 mM octane sulfonate adjusted to pH 4.0–4.5) was delivered at 0.6–0.8 ml/min by a Waters model 510 pump. Amines were detected with a Waters 460 detector with a glassy carbon electrode maintained at +0.65 V relative to a Ag/AgCl reference electrode. Values are presented as pmol/mg protein.

2.6. Data analysis

PPI was calculated as a percent reduction in startle magnitude on PREPULSE trials compared to PULSE trials (%PPI=100×(amplitude on PULSE trial−amplitude on PREPULSE trial)/amplitude on PULSE trial). Startle data were analyzed using repeated measures ANOVAs, with strain as the between-subject factor, and treatment, trial type and trial block as the within-subject factors. For behavioral measures, alpha was 0.05. HPLC data were analyzed by repeated measure ANOVAs for each chemical in each brain region, using strain, treatment and time point as between-subject factors. When no significant effect of time or time interaction was noted, data were collapsed across time points. Initial comparisons were completed for SDH vs. LEH rats, with separate comparisons made for vehicle vs. APO and vehicle vs. AMPH; data from F1s were included where significant main or interaction effects were detected for SDH vs. LEH rats. For its most conservative use, alpha was adjusted to 0.002 to correct for multiple comparisons (0.05/(6 chemicals×4 brain regions)). Post hoc comparisons of significant interaction effects and relevant main factor effects were conducted using Fisher's Protected Least Significant Difference (PLSD) and one-factor ANOVA tests.

3. Results

Behavioral data are seen in Fig. 1. For ease of presentation, unless otherwise stated, several normal parametric effects can be assumed to be statistically significant in all startle analyses: e.g. effects of trial block on startle magnitude and of prepulse intensity on PPI. SDH and LEH rats exhibited comparable levels of PPI after vehicle injection, and PPI was reduced in both strains after treatment with either APO or AMPH. However, the PPI-disruptive effects of both APO and AMPH were significantly greater in SDH vs. LEH rats, consistent with our

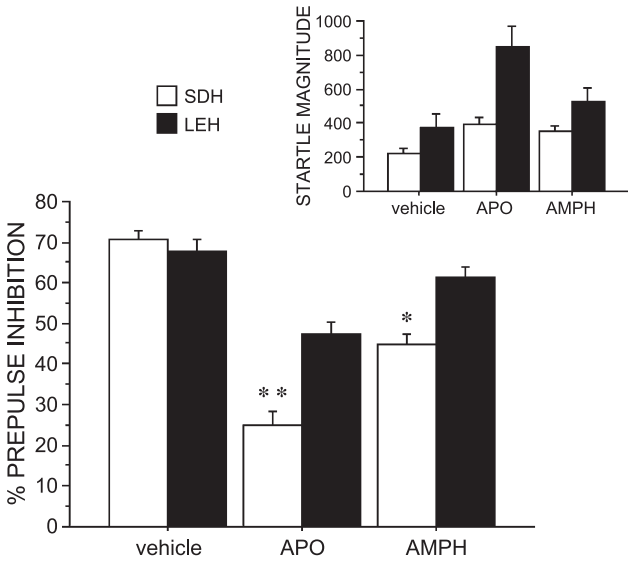

Fig. 1. Percent PPI (S.E.M.) in SDH and LEH rats after treatment with saline, APO (0.5 mg/kg sc) or AMPH (4.5 mg/kg sc). **p<0.005, *p<0.01, SDH<LEH, after significant strain×drug interaction by ANOVA. Data are collapsed across prepulse intensities and trial blocks. Inset: startle magnitude (S.E.M.) on pulse alone trials, collapsed across trial blocks.

previous reports (Swerdlow et al., 2002, 2003, 2004a,b). ANOVA of PPI revealed a significant main effect of strain (F=6.29, df 1,21, p=0.02) and drug (F=45.74, df 2,42, p<0.0001), and a significant interaction of strain×drug (F=7.24, df 2,42, p=0.002). There were no significant interactions of prepulse intensity×drug or strain and no significant three-way interactions. Post hoc comparisons revealed significantly higher PPI levels in LEH vs. SDH rats after treatment with APO (p<0.005) and AMPH (p<0.01).

Strain differences were also detected in other startle measures: APO-potentiated startle—but not AMPH-potentiated startle—was greater in LEH vs. SDH rats, while APO-stimulated NOSTIM activity was greater in SDH rats compared to LEH rats. ANOVA of startle magnitude during blocks 1 and 4 revealed main effects of strain (F=4.87, df 1,22, p<0.05), drug (F=11.93, df 2,44, p<0.0001) and block (F=91.83, df 1,22, p<0.0001), but no significant interactions of strain×drug or strain×drug×block. ANOVA of startle magnitude during blocks 2 and 3 (Fig. 1, inset) revealed main effects of strain (F=4.41, df 1,2, p<0.05), drug (F=18.97, df 2,44, p<0.0001) and block (F=7.44, df 1,22, p<0.015), and a significant interaction of strain×drug (F=4.79, df 2,44, p<0.015). Post hoc comparisons revealed that APO significantly increased startle magnitude in both SDH (p<0.0001) and LEH rats (p<0.0001), while AMPH increased startle magnitude only in SDH rats (p<0.002). Startle magnitude in LEH rats exceeded that in SDH rats after APO (p<0.015), but not after vehicle or AMPH. ANOVA of NOSTIM values revealed main effects of strain

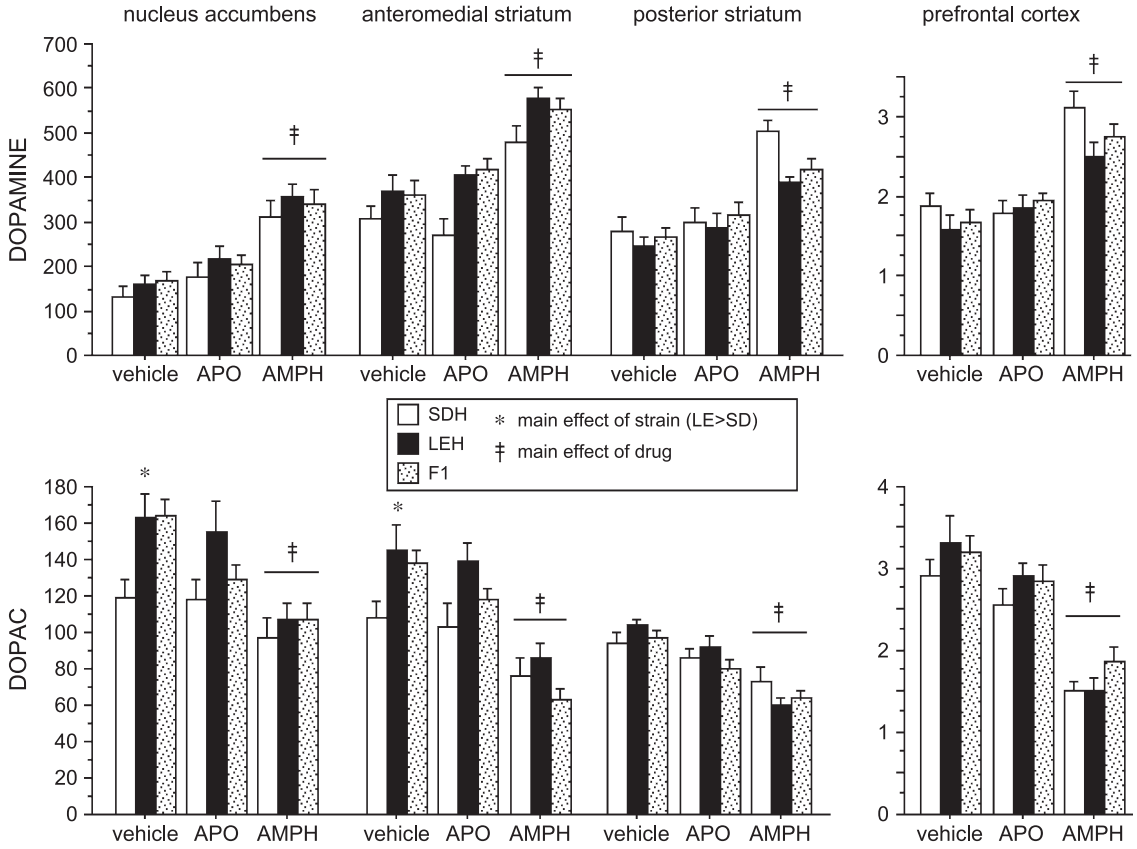

Fig. 2. DA and DOPAC in SDH, LEH and F1 rats in forebrain regions (mean pmol/mg protein±S.E.M.). Data are collapsed across time points 10, 20, 30 and 60 min after drug injection, based on little or no impact of time on patterns of drug effects across strains.

(F=8.23, df 1,22, p<0.009) and drug (F=14.60, df 2,44, p<0.0001), and a significant strain×drug interaction (F=8.47, df 2,44, p<0.001). Post hoc comparisons revealed that APO significantly increased NOSTIM levels in SDH (p<0.0001) but not LEH rats, while AMPH did not increase NOSTIM values in either strain. NOSTIM values in SDH rats exceeded those in LEH rats after APO (p<0.006), but not after vehicle or AMPH.

Neurochemical data are seen in Figs. 2–5. In general, SDH vs. LEH strains differed in basal (post-vehicle) levels of DOPAC (Fig. 2, bottom) and HVA (Fig. 3, top), but not in drug-induced changes in any indices. When striatal regions were grouped (NAC, AMS, PS), compared to SDH rats, LEH rats had significantly higher levels of DOPAC and HVA. The strain difference in DOPAC levels was evident across the three striatal regions, but only reached significance for the NAC and AMS (Fig. 2, bottom: main effect of strain, p<0.009; post hoc strain effects: AMS-p=0.03, NAC-p<0.015). The strain difference in HVA levels was also evident across the three striatal regions, but like DOPAC, reached significance only for the NAC and AMS (Fig. 3, top: main effect of strain, p<0.003, strain×region interaction p<0.035; post hoc strain effects: AMS-p<0.006, NAC-p<0.004). HVA levels in the mPFC were also elevated in LEH vs. SDH rats (p<0.001). No strain differences or strain×region interactions were detected in 5HT or 5HIAA (Fig. 4).

Basal neurochemical levels in F1 rats were examined in the three indices that differed significantly between SDH and LEH rats (striatal DOPAC and HVA, and mPFC HVA). In both striatal and mPFC DOPAC levels, F1 rats exhibited values intermediate between SDH and LEH rats, while in measures of striatal HVA, F1 levels were comparable to, or exceeded, those of LEH rats, and were significantly greater than those in SDH rats in the NAC and AMS (Figs. 2 and 3).

Consistent drug effects on DAergic and 5HTergic indices were also observed, over the time course used for PPI testing (typically up to 30 min post-drug) (Fig. 5). Grouping striatal subregions revealed that AMPH significantly increased DA and MeTYR levels, and significantly reduced DOPAC and HVA levels (and DA turnover, not shown) (Figs. 2 and 3; time course seen in Fig. 5). These patterns were consistent across all striatal subregions, while AMPH effects on DAergic indices in the mPFC were limited to increased DA and DOPAC in this study. 5HT levels were also increased by AMPH in all brain regions, including the mPFC (Fig. 4). In contrast to AMPH, APO produced only one consistent change in DAergic indices, significantly reducing HVA levels (Fig. 3, top). APO-induced increases in 5-HIAA across the grouped striatal regions (p<0.03) achieved only uncorrected levels of significance in the NAC (p<0.008).

Importantly, no clear patterns emerged in which SDH rats exhibited greater sensitivity than LEH rats to drug-induced changes in these neurochemical indices. Of the 24 possible

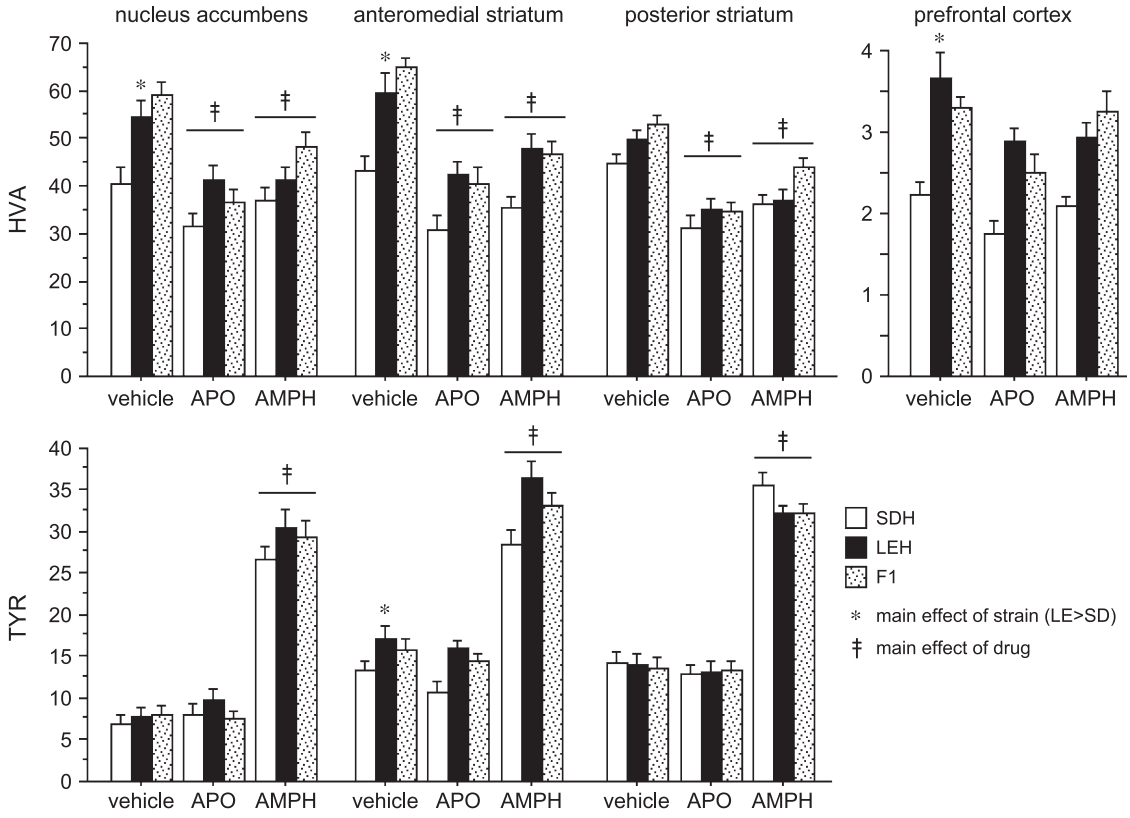

Fig. 3. HVA and MeTYR in SDH, LEH and F1 rats in forebrain regions (mean pmol/mg protein±S.E.M.). Data are collapsed across time points 10, 20, 30 and 60 min after drug injection, based on little or no impact of time on patterns of drug effects across strains.

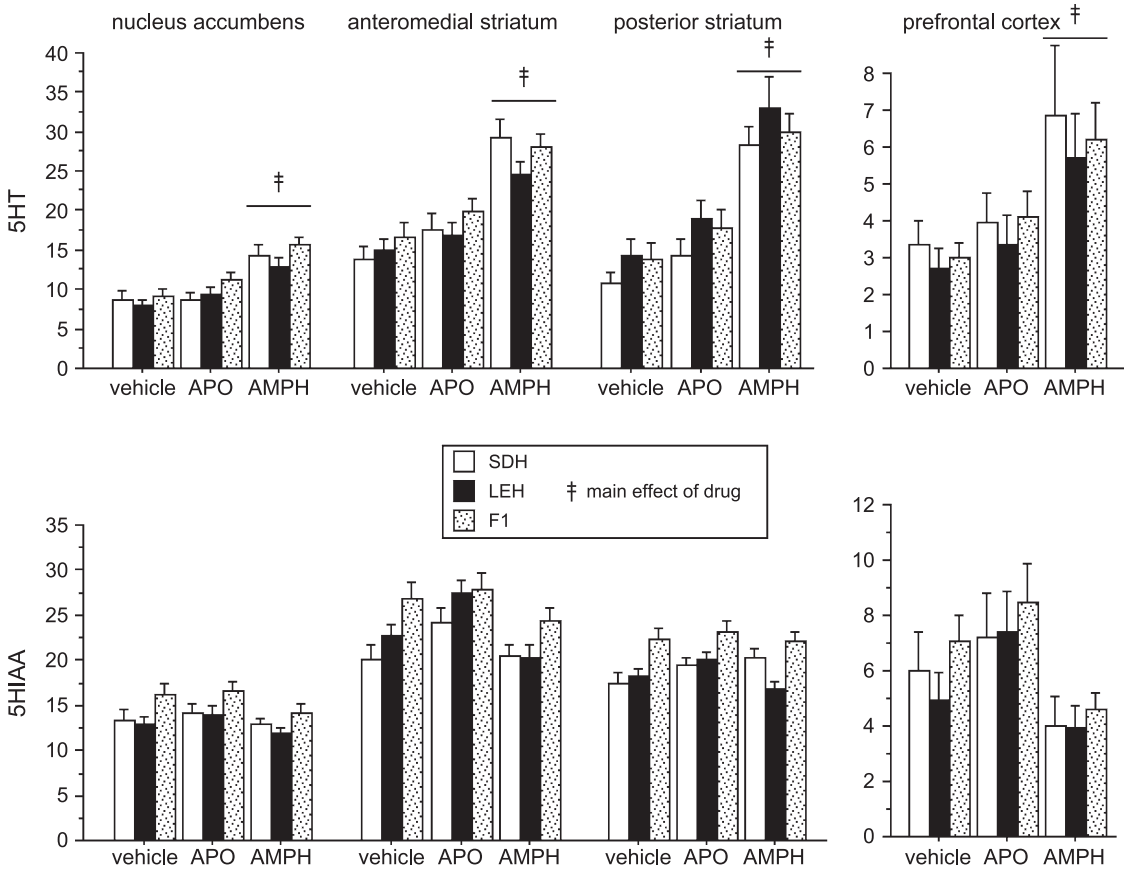

Fig. 4. 5HT and 5HIAA in SDH, LEH and F1 rats in forebrain regions (mean pmol/mg protein±S.E.M.). Data are collapsed across time points 10, 20, 30 and 60 min after drug injection, based on little or no impact of time on patterns of drug effects across strains.

strain×drug interactions for each of the two active drugs, the strain×drug interaction reached uncorrected statistical significance only for one drug (AMPH) for one measure: DOPAC levels in the PS ($p<0.04$). This interaction reflected a greater reduction in DOPAC in LEH vs. SDH rats (Fig. 2). Interestingly, mean AMPH-induced reductions in DOPAC for F1 rats (32.52 pmol/mg protein) was intermediate between SDH rats (20.47 pmol/mg protein) and LEH rats (44.04 pmol/mg protein) (Fig. 2). The only pattern that was at least directionally consistent with the behavioral findings was SD>LE AMPH-increased DA levels in the PS; for this pattern, the strain×drug interaction failed to reach significance ($p<0.08$), but DA levels were significantly higher in SD vs. LE rats after AMPH ($p<0.0007$) and not after vehicle (p>0.38). No significant strain×drug interactions were detected for 5HT or 5HIAA, either within the striatum (all F's<1.63) or mPFC (all F's<1) (Fig. 4).

4. Discussion

The present behavioral findings confirm that compared to LEH rats, SDH rats exhibit greater sensitivity to the PPI-disruptive effects of direct (APO) and indirect (AMPH) dopaminergic activation. We have previously demonstrated that this strain difference is a heritable trait that appears to have a relatively simple mode of genetic transmission. Clearly, this heritable difference must reflect differences in brain mechanisms, and the present investigation represents an initial attempt to elucidate these mechanisms. The ultimate aim of this line of inquiry is to identify the brain basis for genetically conferred differences in a vulnerability to the disruption of sensorimotor gating.

Neurochemical findings suggest that the observed strain differences in PPI "disruptability" do not reflect differential drug sensitivity per se. In other words, the primary indices of drug-induced changes in DAergic or serotonergic function (AMPH- or APO-induced changes in transmitter content, release or metabolism) did not differ in any consistent pattern in SDH vs. LEH rats; certainly, no finding emerged among these indices that could easily account for the magnitude of the differential behavioral responses in these strains.

Drug effects, and strain differences, were also evident in measures of startle magnitude and NOSTIM activity. Findings of strain differences in drug effects on startle measures, other than PPI, have not been consistent across reports, and do not correspond in any simple fashion to the observed differences in PPI sensitivity (Swerdlow et al., 1997, 2000b, 2001b, 2002, 2004a,b). For example, in the

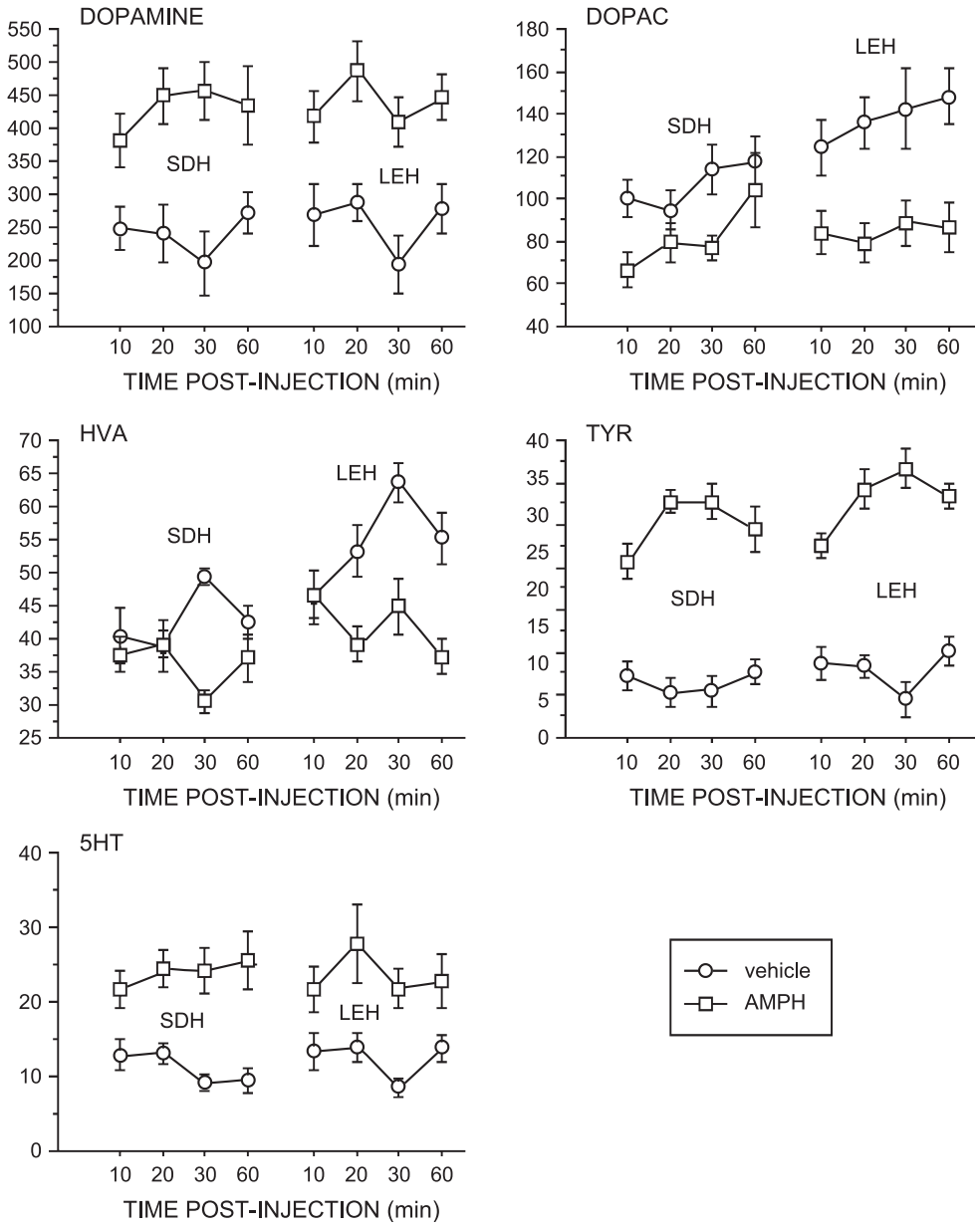

Fig. 5. Time course of AMPH effects on DA, DOPAC, HVA, meTYR and 5HT in striatal regions (mean pmol/mg protein±S.E.M.) in SDH and LEH rats.

present study, APO potentiated startle magnitude in LEH rats more so than in SDH rats, while AMPH potentiated startle in SDH rats but not in LEH rats, and yet both drugs had more potent PPI-disruptive effects in SDH vs. LEH rats. These findings are consistent with a number of reports suggesting that—except at extreme levels—changes in startle magnitude do not necessarily predict changes in PPI (cf. Swerdlow et al., 2000a).

In the present study, neurochemical measures were collected from rats that did not undergo startle testing. It is possible that neurochemical evidence for strain differences in DA agonist sensitivity might have been detected in rats that had also experienced the stress of confinement and exposure to 118 dB(A) white noise bursts. Compared to SD rats, LE rats are reportedly less responsive to cold stress (Riesselmann et al., 1992), though no evidence is available to predict differential strain sensitivity to audiogenic or restraint stress. Humby et al. (1996) reported evidence from microdialysis that startle stimuli suppress NAC DA release in Lister hooded rats, and that this effect is inhibited by prepulses; using different prepulse parameters in Sprague–Dawley rats, they reported PPI that was sensitive to disruption by an NMDA antagonist. It is not known whether PPI of startle-induced DA suppression is disrupted by DA agonists; if it were, then we would predict that after administration of a DA agonist, startle stimuli, with or without prepulses, would reduce NAC DA release. A strain difference in DA agonist sensitivity would then be reflected by differences in prepulse effects on NAC DA levels: strains more sensitive to DA agonists would exhibit less prepulse

inhibition of startle-induced suppression of NAC DA release, i.e. more of a reduction in NAC DA release after prepulse+startle stimuli. However, in addition to not knowing whether DA agonists disrupt this neurochemical effect of prepulses, we also do not know how such effects might interact with the stimulus-independent neurochemical effects of these drugs or differences in basal levels of DA or DA turnover, or what cause–effect relationship might account for a reduction in prepulse effects on NAC DA release in the context of reduced PPI of startle behavior. Lacking this information, we opted to first try to understand strain differences in DA agonist sensitivity outside of the context of audiogenic or restraint stress. Future studies are planned to pursue these more complex designs using in vivo microdialysis in forebrain DA terminal fields.

Differences were observed in the basal "tone" of several DA-related indices in SDH and LEH rats that warrant consideration. Greater DOPAC and HVA levels across forebrain regions in LEH rats suggest that these rats may have elevated basal levels of DA turnover compared to SDH rats; intermediate levels of DOPAC were detected in F1 rats. Because no SDH vs. LEH differences are consistently observed in basal levels of PPI, one might speculate that brain mechanisms involved in the DAergic regulation of PPI may have "compensated" in some fashion for elevated DA turnover in LEH rats, in a manner resulting in a decreased sensitivity to the gating-disruptive impact of high basal DA tone. Conversely, the "set point" for the DAergic regulation of PPI in SDH rats may be influenced by the relatively lower levels of basal DA turnover, in a manner resulting in an increased sensitivity to the gating-disruptive effects of DA agonists. Of course, given the present findings, it is merely speculation that "compensatory mechanisms" lead to reduced DA receptor sensitivity in LEH rats (or increased sensitivity in SDH rats). However, some indirect evidence may support this hypothesis.

Strain differences in PPI sensitivity occur after treatment with either direct or indirect DA agonists. While it is important to consider all possible mechanisms that might account for this fact, the most parsimonious explanation is that these strains differ at, or "beyond", the level of the DA receptor, since this is the common substrate impacted by APO (directly) and AMPH (indirectly, via increased DA release). While outbred rats (including SD and LE rats) differ in the genomic organization of their D2 receptor loci (Luedtke et al., 1992), unpublished findings suggest that differences in forebrain D2 density or affinity cannot easily account for strain differences in PPI APO sensitivity (R. Luedtke, unpublished observation). "Compensation" to differential levels of D2 receptor stimulation within the NAC (that might occur in LEH vs. SDH rats, based on the present findings) is thought to involve postsynaptic and perhaps intracellular processes, including changes in the cAMP signaling cascade (Koeltzow et al., 2003; Culm et al., 2004; Culm and Hammer, 2004). Both DA receptors and coupled G-proteins compensate to tonic levels and phasic changes in DA receptor stimulation; alterations in G-protein-mediated intracellular signaling in transgenic mice are associated with differing basal levels of PPI (Gould et al., 2004).

While there are numerous substrates "beyond" the DA receptor that might contribute to the observed heritable differences in PPI sensitivity, our preliminary findings (in preparation) suggest that SDH and LEH rats do not differ in the PPI-disruptive effects of GABA receptor blockade in the ventral pallidum, the next synapse "downstream" from the DA receptor in PPI-regulatory circuitry (Swerdlow et al., 2001a). Anatomically, this narrows the most likely locus for this substrate to somewhere within the medium spiny striato-pallidal neuron: distal to the DA receptor, but proximal to the pallidal GABA receptor. Thus, our present efforts to identify the substrate for this heritable difference are focused on signal transduction and related intracellular mechanisms within ventral striatal medium spiny neurons.

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