Motor and locomotor responses to systemic amphetamine in three lines of selectively bred Long-Evans rats

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ABSTRACT

The goal of the study was to measure spontaneous and amphetamine-induced motor and locomotor activity in three selectively bred lines of male Long-Evans rats. The number of 50 kHz ultrasonic vocalizations (USVs) emitted in response to heterospecific play with human hand ("tickling") had been measured daily in these lines of rats from 21 to 24 days of age, as a criterion for dividing them into high vocalizing line, low vocalizing line, and random breeding and testing lines. This study sought to determine whether selection of rats based on their affective social-vocalizations also had effects on their locomotor performance and sensitivity to amphetamine. In this study adult animals from the 25th generation (with no further selection) were tested. The results showed that rats, which were selectively bred to emit high numbers of 50 kHz vocalizations, also exhibited elevated levels of spontaneous locomotor activity. After systemic injection of D-amphetamine (1.5 mg/kg), the level of motor and locomotor activity significantly increased further in all the lines as compared to saline controls. The horizontal and vertical activities and the distance covered by rats of the high line, both at the baseline and after amphetamine challenge, were significantly higher than those of the low line animals in absolute scores but not as proportion of relevant saline controls. Since appetitive 50 kHz USVs and locomotor activity are both dependent on the activity of the dopamine system, it is concluded that selection of rats based on the expression of their positive emotional state is also selecting other features than vocalization, namely locomotor behavior. This may help explain why these animals are relatively resistant to depressogenic manipulations.

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1. Introduction

Rat 50 kHz ultrasonic vocalizations (USVs) have been shown to reflect a positive affective state, whereas 22 kHz USVs reflect a negative affective state. Rewarding stimuli have been shown to selectively elevate rates of hedonic 50 kHz USVs, whereas aversive stimuli reduce rates of 50 kHz USVs. Alternative non-hedonic interpretations of these vocalizations are not supported by the available data (Knutson et al., 1999, 2002; Panksepp and Burgdorf, 2000, 2003; Burgdorf et al., 2007, 2008; Burgdorf et al., in press). Conversely, rat 22 kHz USVs are primarily elicited by aversive stimuli such as social defeat and footshock, and have been shown to reflect a negative emotional state consistent with anxiety (Brudzynski, 2001, 2007; Knutson et al., 2002; Brudzynski and Holland, 2005; Burgdorf et al.,

2008). In agreement with these findings, replay of 50 kHz or 22 kHz vocalizations to the recipient rats caused their approach to the 50 kHz calls and behavioral inhibition in response to 22 kHz calls (Wöhr and Schwarting, 2007).

Rats selectively bred for differential rates of USVs show alterations in anxiety and depressive-like behaviors as well as reward sensitivity (Brunelli, 2005a, b; Burgdorf et al., 2005, 2009). For instance, rats selectively bred for high rates of infantile distress USVs show an anxious and depressed phenotype (Brunelli and Hofer, 2007). Adolescent rats selectively bred for high rates of hedonic 50 kHz USVs show a stress resilient phenotype and low rates of aversive 22 kHz USVs, whereas rats bred for low rates of hedonic 50 kHz USVs show a depression prone phenotype and higher rates of aversive 22 kHz USVs (Harmon et al., 2008; Burgdorf et al., 2005, 2009). In addition, animals bred for high rates of hedonic 50 kHz USVs show increased sensitivity to the rewarding effects of sucrose (Burgdorf et al., 2009).

The mesolimbic dopamine systems has been shown to be functionally implicated in the generation of hedonic 50 kHz USVs (Burgdorf, et al., 2007), and the mesolimbic cholinergic system is functionally involved in the generation of aversive 22 kHz USVs

(Brudzynski and Barnabi, 1996; Brudzynski et al., 1998). Both, systemic and intra-accumbens shell injections of amphetamine robustly elicit 50 kHz USVs, and these effects are block by pretreatment with dopamine receptor antagonists (Wintink and Brudzynski, 2001; Thompson et al., 2006). In addition, dopamine receptor antagonists or 6-OHDA lesions of the mesolimbic dopamine system have been shown to reduce rates of 50 kHz USVs elicited in sexual encounters (Ciucci et al., 2007, 2009). In contrast, release of acetylcholine in the medial, periventricular hypothalamic–preoptic and other forebrain regions (medial cholinoceptive vocalization strip) can trigger 22 kHz vocalizations (Brudzynski, 2001, 2007, 2009).

Locomotor behavior is a well-established behavioral assay to examine exploratory activity controlled by the mesolimbic dopamine system (Mogenson, 1991). Amphetamine-increased locomotor activity is empirically linked with the functions of the ascending dopamine system and not nearby norepinephrine networks (Costall and Naylor, 1973; Roberts et al., 1975). More recent results, however, indicated role of α1 adrenergic receptors in the prefrontal cortex in the D-amphetamine-induced release of dopamine in the nucleus accumbens (Darracq et al., 1998). The ascending mesolimbic system from the ventral tegmental area to the nucleus accumbens appeared to be the critical system for the dopamine mediated psychomotor activation that characterizes all appetitive behaviors 'energized' through dopamine-mediated reward seeking (Ikemoto and Panksepp, 1999; Alcaro et al., 2007), as affirmed by the robust locomotor arousal effects of amphetamine in the nucleus accumbens (Pijnenburg and Van Rossum, 1973; Pijnenburg et al., 1975a, b; Kelly et al., 1975; Costall et al., 1976; Groves and Rebec, 1976).

More recently, quantitative measurements of rat ultrasonic vocalizations have been proposed as a more reliable endogenous index of animals' positive affective states than general behavioral measures such as locomotor activity (Knutson et al., 2002; Panksepp, et al., 2002; Brudzynski, 2007, 2009, 2010; Burgdorf, et al., 2008). This agrees with recent results that the magnitude of the sensitization of the cocaine-induced 50 kHz vocalization correlates positively with sensitization of locomotor activity in the same rats (Mu et al., 2009).

Thus, D-amphetamine may be used as an indirect probe to evaluate the underlying dopamine-dependent functional differences among the above-mentioned lines of rats selectively bred for high and low 50 kHz USVs. We have recently demonstrated that intraaccumbens amphetamine has different effects in the high and low lines of rats of the 20th generation selected for 50 kHz calls. While amphetamine dramatically increased the number of 50 kHz calls in the high line, the low line rats exhibited no differential sensitivity to this agent as compared to randomly bred animals (Brudzynski et al., 2010). Our previous work with these selected lines of rats has also revealed differences in motor and motivated behaviors suggestive of temperamental differences. The high line rats showed (i) more entries into the center area in an open field activity test, (ii) an elevated preference for a sucrose solution over water, and (iii) were less aggressive than the random line rats (Burgdorf et al., 2009). Although some results indicted less rearing in the low line rats than in the random line, and more entries to the center of the open filed by high line rats, the counts of line crossing appeared not to be sensitive enough to reveal differences in locomotor activity among lines (Burgdorf et al., 2009). In the present study, an automated cage with a dense grid of infra red light beams has been used.

Since overall locomotor activity during extended tests remains to be studied in these affectively distinct lines of animals selected for 50 kHz 'tickle'-induced vocalizations, the questions arises whether the rats of these lines will also differ in the amphetamine-induced locomotor activity in a comparable way to the differences already seen with amphetamine-enhanced production of 50 kHz calls.

In sum, the aim of this study was to examine how the selective breeding of rats for their positive-affect indexing appetitive vocalizations influenced their locomotor arousal and sensitivity to systemic amphetamine. Our prediction was that the high-line animals would be more active and more responsive to amphetamine, with the low-line rats exhibiting opposite trends.

2. Materials and methods

2.1. Animals

The study was performed on 66 male Long-Evans rats taken from the three lines of selective breed animals (high, low, and random lines) based on differential numbers of 50 kHz USVs to a controlled social stimulus for four successive days from 21 to 24 days of age. The rats originated from the 25th generation of colony bred at the Animal Facility at Brock University, Ontario, Canada, from pregnant Long-Evans strain females initially purchased from the Charles River (Charles River Laboratories Saint-Constant S.A., St. Constant, Quebec, Canada). Rats were housed in translucent polycarbonate cages ($27 \times 24 \times 20$ cm) with dust-free corncob bedding and black polyvinyl tubing as a hiding place. The bedding was changed twice per week. They had *ad libitum* access to water and pellet food (5012 Rodent Lab Diet, PMI Nutrition International, LLC, Brentwood, MO) and were kept in a temperature-controlled room ($23\ °C \pm 1\ °C$) with a 12:12 h dark: light cycle (lights on at 8:00 am). All rats used in the study were under veterinarian control and were healthy and easy to handle throughout these experiments. Animals' body weights ranged from 330 to 520 g at the midpoint of the tests.

2.2. Selection of rats for breeding

After weaning at 21 days of age, rats were housed individually and tested with a standard "tickle" stimulation (heterospecific play resembling rough-and-tumble play behavior with human hand) for four days (day 21–24). The play procedure has been described elsewhere (Burgdorf et al., 2005; Panksepp and Burgdorf, 2000). In short, rats were tested in a rectangular plastic box ($45 \times 35 \times 20$ cm) with corncob bedding, and received 2-min standard "tickle" stimulation sessions. Each session consisted of four alternating cycles of 15 s of stimulation, followed by 15 s of no stimulation. The "tickling" consisted of rapid finger movements across the rat's back and neck, followed by rapidly and repeatedly turning the animal on its back, and alternating finger movements on their ventral surface for 1–2 s, repeated during 15 s, after which the rat was released for 15 s. The entire procedure repeated in exactly the same way on each animal and by the same researcher, was termed "tickling" and was performed once per day for four days. A condenser microphone (SM-1, Ultrasound Advice, London, England) was placed about 35 cm above the cage floor and all emitted vocalizations were recorded using a bat detector (model S-200, Ultra Sound Advice) with 1:16 ratio of frequency division. The divided recordings were stored on TDK SA-60 audiocassettes using a standard tape recorder (VSC-2001, Intertan Canada, Ltd, Barie, ON). The recorded vocalizations were analyzed off line on the DSP Sona-Graph digital sound processing work station (model 5500, Kay Elemetrics Corp., Pine Brook, NJ). The 50 kHz vocalizations were identified sonographically from the monitor and counted in the 15 s stimulation bouts. Scored rats of the first generation were divided into low line animals (the lowest counts) and high line animals (the highest counts of 50 kHz calls). The third random line consisted of randomly chosen rats from the group of rats, which remained after removing the extreme high or low vocalizers. This procedure was repeated with each successive generation after weaning. High lines rats were always selected among high liners, low line rats among low liners, and the random line rats were randomly selected from the random line animals. For further details, see Burgdorf et al. (2005).

2.3. Drug application

After selection, rats of each generation were kept in the same-sex community cages (2–3 rats per cage). The animals were kept randomly in relation to their individual levels of vocalization but rats of different lines were not mixed together in one cage. After reaching three months of age, rats were treated with D-amphetamine sulfate (dextroamphetamine, Sigma-Aldrich Canada Ltd., Oakville, ON) dissolved in isotonic saline. The first test group of rats (10 rats from each line; total n = 30) was systemically injected with D-amphetamine in order to find the route of delivering the drug that will induce higher increase in locomotor activity. The animals received 1.5 mg/kg i.p. or the 1.5 mg/kg s.c. of the drug in a counterbalanced way. The dose of D-amphetamine was based on our previous studies (Wintink and Brudzynski, 2001). D-amphetamine had significantly increased rat locomotor activity regardless of the route of administration (paired t-test, p<0.001), however, rats injected subcutaneously with D-amphetamine showed significantly higher increase in locomotor activity (25% on average) than rats injected intraperitoneally with the same dose of the drug (t[27] = −5.2, p<0.001). Subcutaneous delivery of amphetamine was used for the remainder of the study. The drug was injected subcutaneously on the back of the rats in a volume of 0.2 ml. Isotonic saline served as control. Injections were spaced by seven-day breaks.

2.4. Measurement of motor and locomotor activity

The second group of 36 rats (12 rats per line) were tested. The low line had n = 10 (two rats were eliminated from the study because of infection). After each injection, rats were returned to their home cages for 10 min, which is a sufficient time for the amphetamine to take effect (Pijnenburg et al., 1975a). After this time, animals were transferred to the activity apparatus housed in the same room. Recording of motor and locomotor activity lasted for 20 min and started immediately after placing rats in the activity cage. The inner walls of the activity cage were cleaned with a spray of 50% solution of isopropyl alcohol and wiped out after each rat. The activity cage of the Varimax Animal Activity Monitor (AcuScan Instruments, Inc., Columbus, OH) was a Plexiglas box 40.5 × 40.5 × 40.5 cm with 16 infrared light beams on both the horizontal x-axis and y-axis, creating a grid of perpendicular light beams across the bottom of the cage for measurement of the horizontal activity. The grid of light beams was positioned 2.5 cm above the floor level. Another set of 16 horizontal infrared light beams was elevated 5 cm above the floor level for measurement of the vertical activity (rearing). This higher level of light beams was broken only if the rat raised its head for olfactory scanning (elevated sniffing) or reared on his hind legs. The number and time of broken light beams by moving animal were transmitted to an analyser and downloaded to a PC computer by Versa Max software (AcuScan). The Versa Max software automatically eliminated repetitive breaking of the same light beam (e.g., scratching movements). Also, the animals were continuously observed during recordings to make sure that they behave normally, do not show stereotypic movements or excessive grooming. Motor activity (rearing and grooming) and locomotor activity of rats were measured by three parameters: (a) horizontal activity equivalent to floor exploratory activity and small body movement activity (e.g., grooming), (b) vertical activity representing rearing and elevated sniffing movements, and (c) total distance (in cm) covered by the rat reflecting the magnitude of the locomotor response.

2.5. Statistical analysis

All data were analyzed using the SPSS statistical package (SPSS, Inc., Chicago IL). For simple paired comparisons t-tests were used. All tests were done in a counterbalanced order and repeated measures ANOVA tests were used to analyze results from all lines in relation to motor and locomotor parameters. Tukey's post hoc test was used where appropriate. Greenhouse–Geisser correction for non-sphericity was used, where it was necessary. One-way ANOVA tests were also used to test differences among the three lines with an additional Kruskal–Wallis nonparametric test to directly compare ratios of the amphetamine-induced increase over saline control for each parameter and line.

3. Results

3.1. Horizontal activity

Horizontal activity measured predominantly total locomotor and exploratory activities at the floor level. Factorial ANOVA ran for all non-injected, saline- and amphetamine-injected rats revealed significant differences among the groups (F[2,62] = 154.5, p<0.001, see Fig. 1). Amphetamine-induced activity was significantly elevated as compared to saline-injected rats (cross-hatched bars versus hatched bars), and higher than those for non-injected animals (cross-hatched bars versus blank bars) for all three lines (t-test, p<0.001 for all comparisons; asterisks in Fig. 1).

One-way ANOVA tests have also demonstrated that the three lines significantly differed in their baselines, both in the spontaneous horizontal activity without the drug (F[2,33] = 3.7, p<0.04, Fig. 1, blank bars) or after saline injection (F[2,33] = 13.4, p<0.001, Fig. 1, hatched bars). The baseline for the high line was at significantly higher level of activity than the baseline of the low line (p<0.04 for no drug condition and p<0.001 for saline condition).

Rats of all lines significantly responded to amphetamine, and the response increased by a comparable ratio over the saline level in both the low and high lines (1.9 and 1.8 over saline control, respectively). This was confirmed by the lack of significant interaction between the line factor (low and high) and drug factor (the three drug conditions, F [2,40] = 2.1, p = 0.14) and by direct comparison of ratios over saline (chi-square [2] = 2.02, p = 0.36, n.s.).
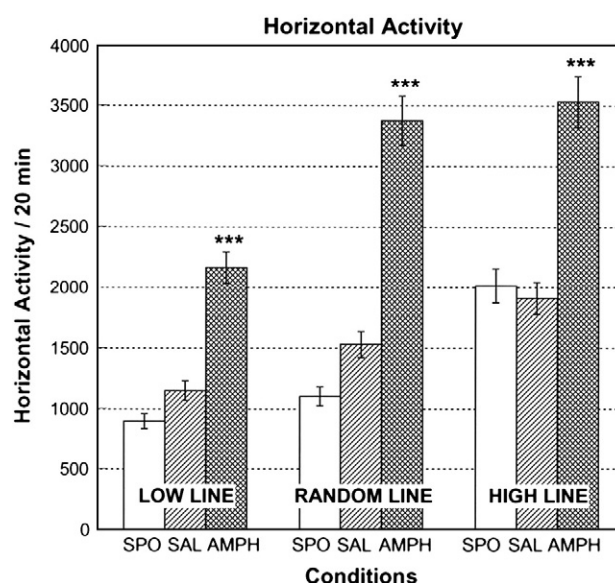

Fig. 1. Horizontal activity (number of broken horizontal light beams during 20 min) was recorded from rats of three lines: Low Line, Random Line, and High Line. The activity of each line was measured in three conditions: non-treatment spontaneous horizontal activity (SPO, blank bars), after systemic saline (SAL, hatched bars), and after 1.5 mg/kg of systemic D-amphetamine (AMPH, cross-hatched bars). Vertical lines represent SEMs. Amphetamine significantly increased the activity over the baseline and saline activities (***, p<0.001). See text for further statistical results (n = 12, except for low line n = 10).

3.2. Total distance covered

The locomotor activity of rats measured by the total distance covered in the activity cage over 20 min revealed similar relationships as the horizontal activity (see Fig. 2). Factorial ANOVA performed for all non-injected, saline- and amphetamine-injected rats revealed significant differences among the groups (F[2,62] = 148.1, p<0.001, Fig. 2). Amphetamine-induced responses were significantly higher than those for saline-injected rats (cress-hatched bars versus hatched bars) or than those for non-injected animals (cross-hatched bars versus blank bars) for all three lines (t-test, p<0.001 for all comparisons; asterisks in Fig. 2).

As confirmed by a one-way ANOVA, rats of all selected lines showed also significant differences in their baseline total distance measures without the presence of the drug (F[2,33] = 16.2, p<0.001, Fig. 2, blank bars), or in total distance after saline injection (F[2,33] = 11.9, p<0.001, Fig. 2, hatched bars). The baseline level of the total distance for the high line was at significantly higher level than the baseline of the low line (p<0.001 for both no drug condition and saline-injected animals).

Rats of all lines responded to amphetamine. The response of high line rats was significantly higher than that of low line animals in absolute measures but not as proportion of saline controls. Although the ratio of the amphetamine response over that of saline was comparable between low line and high line rats (2.1 fold over saline level for both lines), there was a significant interaction between line factor (low and high lines) and the drug factor (three drug conditions, F[2,40] = 5.1, p = 0.01). Direct comparison of the ratios over saline, however, did not show significant differences (chi-square [2] = 2.29, p = 0.32, n.s.).

3.3. Vertical activity

Vertical activity measured rearing and elevated sniffing movements. Factorial ANOVA analysis of the vertical activity of not-injected, and injected rats with saline or amphetamine of all lines showed significant differences among groups (F[2,62] = 30.0, p<0.001, see Fig. 3). Vertical activity after amphetamine was

significantly higher than any control level in all lines as compared to both spontaneous activity (p<0.002 to 0.02) and saline control (p<0.002 to 0.01, asterisks in Fig. 3).

Baseline vertical activity without injections or activity after saline did not differ among lines (Fig. 3, blank and hatched bars). Amphetamine-induced vertical activity among all three lines was similar, although at significantly higher levels than the vehicle level. Although rearing was increased by amphetamine, it proportionally increased over the baseline in each line. Amphetamine-induced vertical activity was 2.0-fold higher than saline control in the random and high lines and 1.7-fold higher than control in the low line. There was no significant interaction between the line factor (low and high) and drug factor (the three drug conditions, F[2,40] = 2.0, p = 0.16). Direct comparison of ratios over saline also did not show significant differences (chi-square [2] = 0.14, p = 0.93, n.s.).

4. Discussion

The general increase in motor activities caused by systemic D-amphetamine in this study is in agreement with well-known behavioral effects of the drug on rat activity (Stein, 1964; Cole, 1967; Ellinwood and Balster, 1974; Groves and Rebec, 1976). The present study finds that the levels of activity significantly differed in most measures among selected genetic lines of rats, which were originally selected not on the basis of their locomotor activity, but on the basis of their vocal expression of emotional state (Panksepp and Burgdorf, 2000; Brudzynski, 2007). This difference in the level of activity pertained both to spontaneous activity without the drug or injection, control activity after saline, as well as to the activity induced by systemic D-amphetamine. Application of D-amphetamine induced higher activity in the high line rats than that in the low line animals in absolute scores, but these activities were not different in relative comparison as proportion of relevant saline controls. This finding may indicate that the selection process, based on the number of emitted appetitive 50 kHz calls (Panksepp and Burgdorf, 2000; Knutson et al., 2002; Wöhr and Schwarting, 2007; Sadananda et al., 2008), has
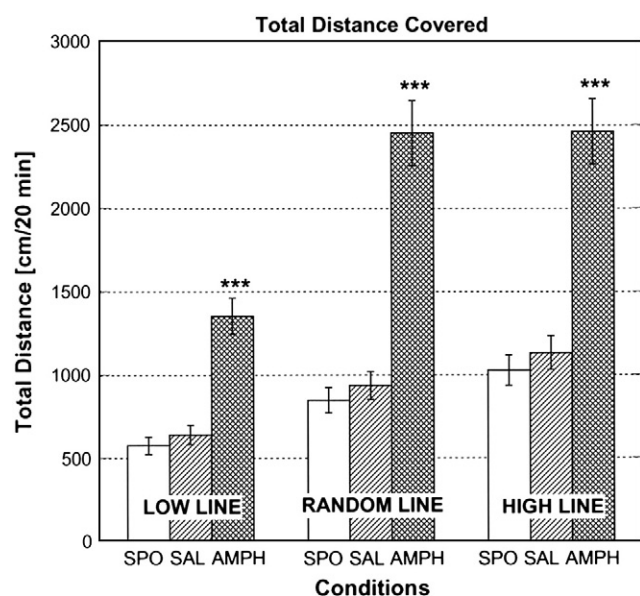
Fig. 2. Total distance (in cm covered per 20 min) was recorded from rats of three lines: Low, Random and High Line. The distance was measured in three conditions: non-treatment spontaneous distance (SPO, blank bars), after systemic saline (SAL, hatched bars), and after 1.5 mg/kg of systemic D-amphetamine (AMPH, cross-hatched bars). Vertical lines represent SEMs. Amphetamine significantly increased the activity over the baseline and saline activities (***, p<0.001). See text for further statistical results (n = 12, except for low line n = 10).
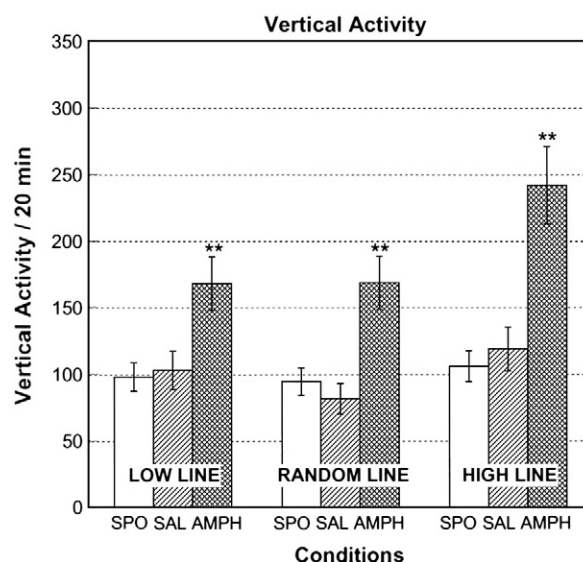
Fig. 3. Vertical activity (number of broken light beams during rearing and raised olfactory exploration) was recorded from rats of three lines: Low, Random and High Line. The vertical activity was measured in three conditions: non-treatment spontaneous activity (SPO, blank bars), after systemic saline (SAL, hatched bars), and after 1.5 mg/kg of systemic D-amphetamine (AMPH, cross-hatched bars). Vertical lines represent SEMs. Amphetamine significantly increased the activity over the baseline and saline activities (**, p<0.002 to 0.02). See text for further statistical results (n = 12, except for low line n = 10).

yielded two broad and distinct temperamental phenotypes represented in the high and low lines, which may constitutionally differ in the general level of their brain dopamine functioning.

Past work has noted that certain strains of rats and mice respond to amphetamine with a high locomotor activity, while responses of others are small by comparison (Segal and Kuczenski, 1987; Hooks et al., 1991; Zocchi et al., 1998). Although, many transmitter systems may modulate this behavior, the differential sensitivity to amphetamine has been attributed to variances in dopamine transmission in the mesocortico-limbic system (Hooks et al., 1991; Ventura et al., 2004). The present results may help explain some of the underlying constitutional features of this phenomenon.

Results of the present study, performed on the 25th generation of selected lines, have further shown that rats of all lines responded to systemic amphetamine as a comparable proportion of the baseline but at the different levels of activity, the lowest one for low line and the highest one for the high line. This finding is in a sharp contrast to results obtained from the same selected lines (at the 20th generation) after intraaccumbens injection of amphetamine and emission of 50 kHz appetitive vocalizations. Amphetamine induced highly significant, 4.3-fold higher than saline level vocal response in the high line, while the low line showed 1.9-fold times increase to the same dose of amphetamine, which did not reach the significance level (Brudzynski et al., 2010). The differences in amphetamine-induced locomotor behavior and vocal behavior may be explained by differences in functions of brain circuitries and their sensitivity to amphetamine.

Although both, emotional expression and locomotor activity depend on the release of dopamine (Thompson et al., 2006; Ciucci et al., 2007, 2009; Brose et al., 1987), there are anatomical and functional differences in the regulation of these behavioral patterns. Dopamine-induced emission of 50 kHz vocalizations is predominantly obtained from the shell portion of the nucleus accumbens (Burgdorf et al., 2001; Thompson et al., 2006), while the stereotypic motor and locomotor activities are ascribed to the core of the accumbens and the dorsal striatum (Vargas-Pérez et al., 2003; Towell et al., 1987). In the rats selectively bred for vocalization, intraaccumbens amphetamine increased 50 kHz calls from the shell of the nucleus accumbens but not from the core or dorsal striatum, a pattern previously seen in unselected animals (Thompson et al., 2006; Burgdorf, et al., 2001). In is noteworthy that results obtained with rats selectively bred for motor performance in a swim test, showed different patterns of results (West et al., 1999). Application of amphetamine directly to the nucleus accumbens in high activity rats showed that swimming activity was significantly and dose-dependently increased from the core of the accumbens, but not from the shell. The low activity rats did not show such difference in their responses (West et al., 1999).

It can be concluded that dopamine regulation of positive emotional expression (by vocalization) and locomotor performance (swimming) depend, at least partially, on different circuitries within the shell and core of the nucleus accumbens and their differential responses to D-amphetamine (Phillips et al., 2003).

In light of this conclusion, the selection of rats based on their vocalizations has also yielded rats with distinct patterns of locomotor performance. Rats with high levels of emission of 50 kHz calls also exhibited elevated levels of locomotor activity as shown in the present study. This observation leads to a general conclusion that selection of rats based on vocal expression of their positive emotional state may also select an array of features depending on the functions of the dopaminergic system with all its components involved and perhaps coordinated in motor, emotive, and other functions. In contrast, the selected lines showed larger differences in sensitivity to amphetamine in emotive measures (emission of ultrasonic vocalization, Brudzynski et al., 2010) than in motor and locomotor parameters.

The present study suggests that our selection process may have augmented a broad spectrum of dopamine-dependent functions although with somewhat different degrees of sensitivity. The motor (vertical) and locomotor (horizontal and total distance traveled) functions were potentiated by the selection process both at the baseline and by a similar proportion after amphetamine-challenge, while the expression of emotionality, as studied elsewhere (Brudzynski et al., 2010), was preferentially potentiated after intraaccumbens application of amphetamine. It may be concluded that selection of rats based on the expression of their positive emotional state also selects for other features dependent on the functions of the dopaminergic system, although with different levels of sensitivity.

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