Effects of intraaccumbens amphetamine on production of 50 kHz vocalizations in three lines of selectively bred Long-Evans rats

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\textbf{Abstract}

Effects of direct injections of amphetamine into the shell of the nucleus accumbens were studied in three lines of Long-Evans rats, two of which had been selected for low and high rates of 50 kHz calls in adolescence in response to a standard social stimulation, and compared to results from randomly selected rats. Injections of amphetamine into the medial shell of the nucleus accumbens significantly increased the number of 50 kHz vocalizations in the high line but not low line, as compared to the random controls. This response was shell specific and antagonized by raclopride. Rats of the high line emitted significantly more frequency-modulated calls, with broader bandwidth and higher mean peak frequency than rats of all other lines. It is concluded that the high line of Long-Evans rats represents animals prone to positively valenced emotional states dependent on endogenous shell dopamine, as compared to the low line animals. Low line rats were less vocal than high and random line rats and not significantly responsive to intraaccumbens amphetamine. Selection of rats on the basis of the number of emitted 50 kHz calls is a useful model for studying brain mechanisms of different emotional phenotypes. The results also indicate that accumbens shell dopamine responsivity may be critical in determining the positive or negative emotional phenotype of the organism.

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\textbf{1. Introduction}

Using selective breeding of laboratory rats for different amounts of ultrasonic vocalization, several studies have demonstrated that the magnitude of ultrasonic signaling is heritable and may signal different types of emotionality both in juvenile and in adult rats [5,6,8,15,22,24]. After many generations of selective breeding of 10-day old rat pups for the rate of social isolation calls in response to maternal separation (aversive calls), low and high vocalizing lines were obtained [5]. Rats of these two lines differed not only in the emission of vocalization but also in many physiological parameters, neurochemical variables, and behavior during their lifespan, from infancy to adulthood [5–7]. For example, rats of the high vocalizing line showed a higher rate of defecation and urination during isolation than the low vocalizing line or the control random line (not selected rats), and both low and high lines had changed autonomic regulation of heart rate [5,7]. Furthermore, rats of these selected lines showed differences in play behavior, which may represent temperamental and emotional difference. Rats originating from the high-vocalizing line in infancy represented a phenotype consistent with an anxious and depressed trait while those originating from low-vocalizing line in infancy showed behavior and autonomic changes consistent with an aggressive type [6]. Thus, selective breeding for infantile vocalization that reflects a negative emotional process (and can be reduced by anxiolectics) produced lines of animals with differences in emotional behaviors and physiological responsivity in adulthood [30]. Thus, the selective breeding resulted in obtaining different “emotional phenotypes”, i.e., lines of rats differing in their average emotional expression and responding.

Studies on adult rats suggested that the emission of a 22 kHz vocalization signals a negative emotional state to conspecifics, which is consistent with the state of anxiety [2,4,11,26]. On the other hand, elevated levels of 50 kHz vocalizations were suggested to reflect positive emotionality and social affect, consistent with a higher hedonic state of joy [11,16,25,26,32,33]. Selective breeding of Long Evans rats (screened at 24 days of age) for heterospecific play-induced 50 kHz calls (“tickling”-induced appetitive calls [13])
for a number of generations produced two lines of high and low vocalizing animals whether tested at the juvenile or adult ages [14,15]. After the first 5 generations, animals of the high vocalizing line emitted significantly more 50 kHz calls and fewer 22 kHz calls than animals of the low vocalizing line [15], and animals of the low line produced more 22 kHz calls and less 50 kHz calls than the high and random lines (non-selected control line) at later generations.

Further studies have demonstrated that the selected lines differed not only in the number of emitted 50 kHz calls, but they represented a broader spectrum of differences in social and emotional behavior [14,22]. In infancy (postnatal day 10–12), the rats of the low vocalizing line (low number of 50 kHz calls at the juvenile stage) showed an increased number of isolation calls compared to controls, and failed to exhibit preference for a maternally-associated odor during the place preference test [22]. In adulthood, animals of the low-vocalizing line (low rate of 50 kHz calls) showed a higher rate of defecation in the open field and “tickling” play tests (heterospecific play), and showed less contacts with conspecifics during a social interaction test, as compared to the randomly selected control animals. On the other hand, adult animals of the high vocalizing line (selected for high numbers of 50 kHz calls) showed more entries into the center of an open field, were more likely to demonstrate preference for sucrose solution, and were less aggressive than the control random line [14]. In general, the rats originating from the high-vocalizing line represented a stress-resistant (or -resilient) phenotype while those originating from the low-vocalizing line presented a stress prone phenotype [14].

These two different approaches to selective breeding based on the number of negative isolation calls in infancy or the number of positive calls at an early juvenile age led to separation of two opposite phenotypes differing in social and emotional behavior. One phenotype is associated with bold animals, showing a high degree of approach behavior and stress resistance, which relates to the low line rats selected for aversive isolation calls and high line rats selected for appetitive 50 kHz calls. The other phenotype contains fearful animals, showing social deficits and anxiety or depressive features, and being prone to stress, which relates to the high line rats selected for isolation calls and low line rats selected for 50 kHz calls.

These studies have demonstrated that selective breeding for vocalization can separate the whole phenotype of rat social and emotional features. This was possible because mammalian vocalization evolved as a signaling system communicating emotional states to conspecifics in social groups and appeared to be an effective index of the signaler’s emotional state and its valence [3,26].

Rats selected for emission of 50 kHz calls appeared to be a useful tool in studying functions of the mesolimbic dopamine system. Production of 50 kHz calls was shown to be mediated and dependent on the function of this system [3,10,38,40]. Systemic and intracaccumbens amphetamine significantly increased the number of emitted 50 kHz calls [1,10,38] while systemic haloperidol or flupenthixol, dopamine antagonists, decreased the number of behaviorally or pharmacologically induced 50 kHz calls [16,40]. Recent studies have demonstrated that increased emission of 50 kHz calls by amphetamine or cocaine could be sensitized with repeated administration of these drugs [1,29]. Furthermore, rats selectively bred for high emission of 50 kHz vocalizations showed a higher magnitude of cocaine-induced sensitization than rats selectively bred for a low level of 50 kHz calls [29].

Thus, rats selectively bred for high and low level emission of 50 kHz calls may be used for investigation of the difference in pharmacological sensitivity of the mesolimbic dopamine system responsible for initiation of 50 kHz vocalizations. Of particular interest is the responsiveness of neurons of the shell of the nucleus accumbens, which was shown to be the most sensitive target in inducing 50 kHz calls by amphetamine [38]. The goal of the present study was to investigate differences in 50 kHz responses to direct intraaccumbens (shell) application of amphetamine in the three inbred lines of Long-Evans rats selected for the level of emitted 50 kHz vocalizations at the juvenile age [14,15]. We hypothesize that these lines of rats would significantly differ in the level of responding to direct amphetamine challenge. Also, particular attention was paid to the type of emitted call, since the dopamine system may be selectively involved in the production of frequency-modulated 50 kHz vocalizations [17]. We also hypothesize that amphetamine challenge would increase production of these frequency-modulated calls.

Using the same lines, we have also verified whether intracaccumbens application of nanogram doses of carbachol, a predominantly muscarinic cholinergic agent, has any effect on emission of 50 kHz calls, as suggested in a recent study [20]. It has been postulated that 22 kHz and 50 kHz vocalizations signal different affective states of the organism [3]. Intracerebral carbachol is known from previous studies to produce aversive type of calls and not 50 kHz vocalizations [2], thus it is important to verify this result. The prediction was that the dopamine-driven production of 50 kHz calls would not be influenced by a low dose of carbachol as reported by Fendt et al. [20].

2. Materials and methods

2.1. Subjects and selective breeding

The Long-Evans rats used in these experiments had been selectively bred into three lines (low, random, and high) depending on the number of 50 kHz calls emitted during heterospecific play (“tickling”) starting at postnatal day 24, after weaning (see for details [13,15]). The original pair of parents for the colony was obtained from Charles River Laboratories, St. Constant, Quebec, Canada. The rats were “tickled” consecutively for four days (2 min/day), and on the fourth day, vocalization recordings were scored for the total number of 50 kHz calls emitted during that playtime. Rats belonging to separate lines were not mixed across the lines. Rats producing the most 50 kHz calls within the high line (HL) were selected for further breeding within that line, while those emitting the least or no calls within the low line (LL) were selected for further breeding in that line. Rats belonging to the third, random line (RL) were not preselected for breeding purposes. Rats from the 20th generation were used for the experiments in this study. Except for the breeding periods, rats of the same sex were pair-housed in standard polycarbonate cages (460 mm × 250 mm) with corn cob bedding and a black polyvinyl tube for hiding. The colony was maintained on a 12/12 h light/dark cycle with water and standard rat chow ad libitum. All experimental procedures were according to the guidelines set by the Canadian Council on Animal Care and were approved by the Animal Care and Use Committee at Brock University.

2.2. Stereotaxic surgeries

At the time of surgery, all rats weighed approximately 350–450 g. Animals were anesthetized by a mixture of ketamine hydrochloride (40 mg/kg i.p., Ketalead, MTC Pharmaceuticals, Cambridge, Ont., Canada) and xylazine hydrochloride (6 mg/kg i.p., Rompun, Bayvet Div. Chemargo Ltd., Etobicoke, Ont., Canada) and were placed in the Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). Chronic stainless steel guide cannulae (640 μm outer diameter, made from 23 gauge needles) were bilaterally implanted in the shell of nucleus accumbens, 1 mm above the intended injection site. The stereotaxic co-ordinates for the nucleus accumbens shell were: A=9.6–10.0 mm from the interaural plane, L=1.1–1.5 mm from the midline, and V=6.6–7.0 mm from the surface of the brain (according to the atlas by Paxinos and Watson [34]). Cannulae were secured to the skull with stainless steel jeweler’s screws and methyl methacrylate resin (Perm, Hygienic Corporation of Canada Inc., St. Catharines, Ont., Canada). After surgery, the openings of the cannulae were plugged with stainless steel wires and rats received 2 ml s.c. warmed physiological saline, an injection of an analgesic ketoprofen (2 mg/kg s.c., Anafen, Merial Canada Inc., Baytril, Bayer Inc., Toronto, Ontario, Canada), and were allowed to recover for 7 days.

2.3. Intracerebral injections and drugs

Animals were bilaterally injected with a 30 gauge stainless steel injection cannulae (310 μm outer diameter) connected to a Hamilton constant rate microsyringe (CR-700-20) at a rate of 0.2 μl/min with a volume of 0.2 μl injected each side. For other details of the injection procedure, see [38]. D-Amphetamine sulfate (Dex-traupharm, Sigma-Aldrich Canada Ltd., Oakville, Ont., Canada) was dissolved...
in a sterile isotonic saline and injected in a total dose of 7 μg. Raclopride l-tartrate (Sigma-RBI, Oakville, Ont., Canada), a dopamine D2 antagonist, was dissolved in isotonic saline and injected in a dose of 7 μg, close to an equimolar quantity to the dose of amphetamine. Raclopride was given unilaterally in order to minimize the damage in the double-injection design. Carbachol (carbamylcholine chloride, Sigma-Aldrich Canada Ltd.) was dissolved in sterile isotonic saline and injected intracerebrally in the dose of 4 or 20 ng. Saline served as a control vehicle. Injections of all drugs were counterbalanced across lines and drug conditions. Each animal was injected into the brain once, except in the double injection design, where amphetamine and raclopride were injected twice, and saline four times (as a pretreatment and double saline–saline control). One brain site was injected only two times for studying amphetamine effects, three times for studying low dose carbachol treatment and double saline–saline control). One brain site was injected only two times for studying amphetamine effects, three times for studying low dose carbachol effects, and five times in the double injection design.

2.4. Histological procedure

After the experiments, animals were euthanized by an overdose of barbiturate (sodium pentobarbital, Euthanyl, Vetoquinol N-A, Lavaltrie, Quebec, Canada) and the injection sites were marked with an intracerebral injection of diluted India ink solution. Brains were transcardially perfused, postfixed in 10% formalin solution, and sectioned with a freezing microtome (Cryo-Histomat, Hacker Instruments and Industries, Fairfield, NJ). Standard 40–50 μm histological sections were stained with thionine, analyzed under the microscope and the injection sites were localized using India ink deposits as an indication of the injection site. The localized sites were mapped on the coronal sections of the rat brain from the stereotactic atlas by Paxinos and Watson [34]. Other histological details were described elsewhere [38].

2.5. Recording and analysis of vocalization

Animals were handled and adapted to the manipulations during injections for four days. Right after intracerebral injection, rats were placed into a clean polycarbonate cage (460 mm × 250 mm) covered by a metal grate. The recording condenser microphone (model CM10/CMPA, Avisoft Bioacoustics, Berlin, Germany) was mounted on the top of the cage with an adhesive tape and was aimed at the recording cage. The recording cage was cleaned with alcohol spray after each animal to remove olfactory residues of the other rats. Each rat was recorded for five minutes at a sampling rate of 500 kHz in 16 bit format and the recording session commenced approximately one minute after the removal of the injection cannula. All recordings were done using Avisoft Bioacoustics software (Avisoft Recorder NI-DAQMX) and stored on the hard drive of a PC computer (Dell Precision 390). Then, the files were permanently stored on CDs for re-analysis. The acoustic files were analyzed off line using the Avisoft SAS-Lab-Pro program (using filter 0–250 kHz and FFT with resolution of 1.95 kHz), and the number of 50 kHz calls, peak frequency, duration of individual calls, bandwidth, and sonographic (spectrographic) structure of vocalizations were recorded. Based on the sonographic shape of frequency changes over time, the 50 kHz calls were categorized into the following types: (a) flat calls having sound frequency relatively stable in time and not changing more than 10 kHz, (b) step-trill calls having sound frequency modulated in a complex form of a sudden jump up in the frequency followed by a modulated trill consisting of 2–5 sine-wave fluctuations, and (c) other frequency modulated calls with sound frequency showing variable changes (e.g., sharp increase or decrease, or complex frequency changes).

2.6. Statistical analysis

All results were analyzed by SPSS statistical software (SPSS Inc., Chicago, IL). Univariate or repeated measures factorial ANOVA were used, where appropriate, with Tukey’s HSD post hoc tests. For additional paired analyses, a Wilcoxon matched-pairs signed rank test was used. The critical level of significance was p < 0.05.

3. Results

3.1. Effects of amphetamine

Direct injection of 7 μg of amphetamine into the shell of the nucleus accumbens of Long-Evans male rats caused an almost 4-fold average increase (all lines together) in the number of emitted 50 kHz vocalizations by the animals (Fig. 1). Factorial ANOVA showed a significant line effect (F[2,15] = 13.84, p < 0.001), significant drug effect (F[1,15] = 13.32, p < 0.002) and significant interaction between the drug and the line effects (F[2,15] = 4.65, p < 0.03). The different lines of rats responded differentially to the same dose of amphetamine challenge. While the LL animals did not respond significantly to accumbens (shell) amphetamine, the other two lines, RL and HL, showed significant increases in the number of calls as compared to saline control (p < 0.01 for both RL and HL). Moreover, the number of emitted 50 kHz calls by particular lines after amphetamine significantly differed from each other (F[2,15] = 10.34, p = 0.002). The RL rats called almost twice more times than the LL animals (p < 0.01) and the HL rats emitted 3.4 times more calls than RL rats (p < 0.01) and 6.4 times more calls than LL rats (p < 0.001) after amphetamine injection. At the same time, there was no significant difference among the saline controls among all three lines and the emitted 50 kHz vocalizations were at or below 4 calls/5 min in each of the lines (F[2,15] = 1.58, p = 0.24, n.s.).

3.2. Effects of raclopride

In order to verify the drug-specific effects of amphetamine, an additional 7 rats from the high line were unilaterally injected with 7 μg of amphetamine into the shell of the accumbens and pretreated with saline or with D2 dopamine receptor antagonist raclopride in the same cannula. Injection of amphetamine pretreated with saline induced an average of 20.8 50–kHz calls/5 min, while the same dose of amphetamine pretreated with an equimolar quantity of raclopride reduced this result to an average of 4.1 50-kHz calls/5 min (5 times decrease, paired t-test, t[6] = 3.1, p < 0.02; see Fig. 4B). Thus, raclopride reversed the amphetamine-induced
response to the saline level (4.1 calls/5 min as compared with 3.1 calls/5 min after a double injection of saline-saline control, n.s., see Fig. 4B for further details). Amphetamine-induced response was significantly higher than any other control with raclopride itself, low dose of carbachol (4 ng) or isotonic saline ($F_{[4,24]} = 7.84, p < 0.001$, Fig. 4B).

3.3. Localization of the response

Histological localization of injection sites in the nucleus accumbens has shown that the bilateral injection sites of six rats/line taken for analysis were placed in comparable regions of the shell of the nucleus accumbens with a few sites close to the border of the accumbens core (Fig. 2). Responses induced by amphetamine were structure-specific and injection of the same dose of amphetamine below the shell region failed to induce such a response. Summary of injection sites from five rats of the HL, which received injections into the structures below the shell of the accumbens (olfactory tubercle, ventral pallidum, and islands of Calleja), is illustrated in Fig. 3B. Although rats of the HL were characterized by the highest increase in the number of 50 kHz vocalizations from the shell region, injections into the neighboring olfactory tubercle and ventral pallidium failed to induce significant responses and remained at the level of calling comparable with that for saline (Wilcoxon, $p = 0.43$, n.s., Fig. 3A). Responses induced by injected amphetamine into the accumbens core and structures dorsal to the nucleus accumbens were carefully studied and published elsewhere (injections of amphetamine into the core of the accumbens caused a 3.5 times lower number of 50 kHz calls than from the shell region, while injections of amphetamine above the core to multiple locations of the caudate-putamen failed to induce any response [38]).

3.4. Effects of low dose of carbachol

Direct bilateral injection of 4 ng or 20 ng of carbachol into the shell of nucleus accumbens into the same brain sites as amphetamine did not cause any significant effect on the number of emitted 50 kHz calls (Fig. 4A, bottom graph). The average number of emitted 50 kHz vocalizations after either 4 ng or 20 ng of carbachol remained unchanged both in comparison to the saline controls, as well as in comparison to other lines ($F_{[5,9]} = 2.47, p = 0.96$, n.s., Fig. 4A). The effect of nanogram quantities of carbachol remained similar to that of the isotonic saline. It should be also mentioned that recordings of ultrasonic vocalization did not contain any call having compatible features with those of 22 kHz vocalizations in any of the lines.

3.5. General acoustic analysis of emitted calls

The sound peak frequency of calls emitted after injection of saline or amphetamine was analyzed in RL, LL, and HL rats. The factorial ANOVA revealed only significant line effect ($F_{[2,16]} = 52.98, p < 0.001$, Fig. 5) without drug effect or interaction ($p = 0.99$, respectively). The average sound peak frequency differed significantly among the lines both in the saline condition ($F_{[2,16]} = 42.81, p < 0.001$, Fig. 5) as well as in the amphetamine condition ($F_{[2,22]} = 18.06, p < 0.0005$, Fig. 5). The Tukey’s post hoc test showed that the peak sound frequency of 45.8 kHz in the LL after saline injection was significantly lower than that of the RL after saline (54.5 kHz, $p < 0.03$, Fig. 5, left two cross-hatched bars). In the amphetamine group, a similar tendency appeared but significance was not reached. The LL rats had an average peak frequency of 46.3 kHz after amphetamine while the RL had 50.1 kHz after amphetamine (left two cross-hatched bars, n.s.).
The HL rats showed a more consistent increase in the average peak frequency as compared to that in the RL (Fig. 5). After saline injection, the average peak frequency was 59.8 kHz in HL as compared to 54.5 kHz in the RL ($p < 0.005$, Fig. 5, right two hatched bars). After amphetamine injection, a similar increase was observed among lines and an average peak frequency of 59.8 kHz was recorded in the HL as compared to 54.5 kHz in the RL rats ($p < 0.001$, Fig. 5, right two cross-hatched bars).

In summary, the LL showed lowered and HL higher average peak frequency of 50 kHz calls than the control RL rats ($p < 0.03$ after saline and $p < 0.001$ for combined groups after saline and amphetamine). The peak frequency of the HL was always significantly higher than that of the LL ($p < 0.001$ for saline and $p < 0.0005$ for amphetamine), and therefore remained line-dependent. At the same time, the peak frequency of amphetamine-induced calls did not differ from those after relevant saline injection in all lines (relevant left, hatched, and right, cross-hatched, groups of bars in Fig. 5, $F[1,16]=0.001$, $p=0.99$, n.s.). So, within each line, rats vocalized with almost the same peak frequency regardless of the injected substance.

Analysis of the duration of individual calls across lines and injection conditions, however, did not show any significant differences neither for drug effects ($F[1,16]=2.35$, $p=0.15$ n.s.) nor for line effect ($F[2,16]=0.45$, $p=0.64$, n.s., Fig. 6). Although there were sporadic fluctuations in the average duration of individual calls, neither line nor injection condition statistically differed. Thus, the duration of 50 kHz calls remained relatively constant in all animal lines and conditions.

Finally, analysis of the bandwidth of 50 kHz calls showed significant differences after amphetamine ($F[2,22]=4.55$, $p<0.02$) but not after saline injection ($F[2,15]=0.59$, $p=0.56$ n.s., Fig. 7). Bandwidth of the LL was significantly lower than that of the RL after amphetamine ($p<0.04$, first two cross-hatched bars). The bandwidth of amphetamine-induced calls in the HL was not significantly higher than that of the RL but remained significantly higher than bandwidth of the LL ($p<0.005$, Fig. 7, cross-hatched bars). Similarly to other acoustic parameters, the bandwidth of calls after amphetamine did not differ from the relevant values after saline injection in all lines (left three bars compared to right three bars in Fig. 7, $F[1,15]=0.22$, $p=0.64$, n.s.). Within each line, rats
Fig. 4. Effects of intraaccumbens injections of carbachol in the three lines of Long-Evans rats on production of 50 kHz vocalizations during the first 5 min after injection (50 kHz calls/5 min). (A) Two doses of carbachol (CCh, cross-hatched bars) and saline (Sal, hatched bars) were injected in the low line (LOW), random line (RANDOM) and high line (HIGH) rats. Carbachol was given bilaterally into the shell of the nucleus accumbens in a total dose of 4 ng (4) or 20 ng (20). The injections did not cause significant changes in production of 50 kHz vocalizations. (B) shows results of control experiment in a double intraaccumbens (shell) injection design in the high line rats. Response to amphetamine (Sal + AMPH, 7/9262 g) was significantly reversed by close to equimolar dose (7/9262 g) of raclopride (RAC + AMPH). Raclopride itself pretreated with saline (Sal + RAC), saline (Sal + Sal) or low dose of carbachol (4 ng) pretreated with saline (Sal + CCh) did not increase the number of 50 kHz vocalizations. Vertical lines represent S.E.M.

3.6. Flat versus frequency-modulated (FM) 50 kHz vocalizations

Analysis of sonographic type (flat versus FM) of 50 kHz calls induced by saline or by amphetamine injection was performed in rats of the HL offering high number of calls (Fig. 8). Injection of amphetamine into the shell of the accumbens showed a significant overall increase in both the flat and FM types of calls (Fig. 8). There was a significant effect of amphetamine factor ($F_{1,5} = 9.39, p<0.03$), significant effect of the call type factor ($F_{1,5} = 6.36, p<0.05$), and significant interaction between factors ($F_{1,5} = 8.08, p<0.04$). Thus, the FM calls increased in a different way than FL calls. While the increase in the flat type of calls after amphetamine, compared to saline, did not reach the significance level, the number of the FM calls after amphetamine was significantly higher than that after saline ($p<0.03$). The proportion of flat and FM calls after injection of amphetamine has changed. While the FM calls presented only 5.4% of all calls induced by saline, they constituted 50.8% of calls after amphetamine, a significantly increased proportion (Fig. 8, right two blank bars, Wilcoxon, $p<0.03$).

4. Discussion

Direct injections of amphetamine into the shell of the nucleus accumbens in our adult high-line Long-Evans rats significantly increased the number of emitted 50 kHz vocalizations. The response, however, was line-dependent, which confirmed our hypothesis that the selected lines of rats would differ in the level of responding to amphetamine. While rats of the LL did not significantly respond to amphetamine, rats of the HL emitted 3.4 times more calls than the control RL and 6.4 times more calls than LL animals. The response was specific for amphetamine, reversed by local application of raclopride, and was not present after saline injection or after application of very low (ng) doses of carbachol into the
Fig. 5. Changes of sound peak frequency of 50 kHz calls (in kHz) after injection of saline (hatched bars) or 7 μg of amphetamine (AMPHET, cross-hatched bars) into the shell of the accumbens in low line (LOW), random line (RAND) and high line (HIGH) animals. The line of rats had significant influence on the peak frequency both for saline (p < 0.001) and for amphetamine (p < 0.0005). Rats of the high line had significantly higher peak frequency than random line and low line rats. See text for other statistical details. Error lines represent S.E.M.

Fig. 6. Changes of individual call duration (in ms) of 50 kHz calls after injection of saline (hatched bars) or 7 μg of amphetamine (AMPHET, cross-hatched bars) into the shell of the accumbens in low line (LOW), random line (RAND) and high line (HIGH) animals. The call duration did not differ among lines and drug conditions (p = 0.53). Error lines represent S.E.M.

same brain sites (see [20]). The reversal of the response by raclopride, a D₂ dopamine antagonist, confirms earlier results that D₂ receptor is implicated in the production of 50 kHz calls [38]. Also, in accordance with the prediction, nanogram quantities of carbachol did not induce any response. Although intracerebral carbachol in microgram doses can induce behavioral manifestations and aversive vocalization, it was never reported for nanogram amounts of this drug. This dose seems to be too low to initiate behavioral change.

Fig. 7. Changes of bandwidth of 50 kHz calls (in kHz) after injection of saline (hatched bars) or 7 μg of amphetamine (AMPHET, cross-hatched bars) into the shell of the accumbens in low line (LOW), random line (RAND) and high line (HIGH) animals. The line of rats had significant influence on the bandwidth after injection of amphetamine (p < 0.005) but not after injection of saline (p = 0.39). In the amphetamine group, bandwidth in the high line was significantly higher than that in the low line (p < 0.04) but was not significantly higher than bandwidth of the random line (p = 0.04). See text for other statistical details. Error lines represent S.E.M.

Fig. 8. Number of flat-frequency (FL) or frequency-modulated (FM) 50 kHz calls after injection of saline (SAL) or amphetamine (AMPH) into the shell of the nucleus accumbens of high line rats (n = 6). There were significant differences among the number of emitted calls (p = 0.003). The number of FM calls significantly increased after injection of amphetamine as compared to than after saline (p < 0.03), while the increase in the number of flat 50 kHz calls only approached the significance level (p = 0.062). The proportion of frequency-modulated calls after amphetamine significantly increased (9.4 times) as compared to that after saline (blank bars). See text for further statistical data. The vertical lines represent S.E.M.
4.1. Amphetamine response is localized to shell of the accumbens

The 50 kHz calls were induced by amphetamine predominantly from the shell of the accumbens. We know from our previous work that injections of amphetamine into the accumbens core resulted in a very small response [10,38]. Although the response from the core region was significant, it was 3.5 times smaller than that from the shell region, so only marginally higher than the saline line. Injections of amphetamine to the caudate-putamen or into the ventral structures (olfactory tubercle, ventral pallidum and nearby islands of Calleja) did not induce significant changes in vocalization ([10,38] and the present results). Further analysis of the localization of injection sites within the accumbens shell in the present study revealed that 94% of all sites (34 out of 36) were located within the medial shell region, while the remaining two cannulae were in the central part of the shell (Fig. 2).

The medial shell is defined as the part of the structure between midline structures and the commissura anterior. It has been shown in a self-administration study that the accumbens shell region is functionally heterogeneous and the medial portion of the shell, but not the lateral one, is mediating rewarding effects of a psychostimulant drug MDMA ((±)-3,4-methylenedioxymethamphetamine), an amphetamine derivative [37]. Thus, the robust increase in the number of 50 kHz calls in the HL rats occurred from the region mediating reward.

It has been noticed that local effects of intracerebral amphetamine may differ from structure to structure, as it was recently shown for effects of intracerebral amphetamine in the accumbens shell and the medial preoptic area in relation to mating behavior in female rats [21]. Although the medial olfactory tubercle has also been implicated in the rewarding effects of drugs [37], injections of amphetamine into the olfactory tubercle in the present study did not cause a significant increase in the emission of 50 kHz calls. Amphetamine-induced response from the shell of the accumbens seems to be selectively involved in production of a positive affective type of vocalization.

The critical role of the shell of the nucleus accumbens in responses to psychostimulants was recently documented by electrophysiological and lesion studies. Repeated systemic treatment of mice with amphetamine for 5 days, which showed typical motor sensitization, was followed by an abstinence period and amphetamine challenge. Amphetamine treatment had changed the firing pattern of accumbens shell and core neurons in a subsequent whole-cell recording in ex vivo brain slices from the same animals [27]. While shell neurons showed an initial depression in firing rate for several days, core neurons increased firing rate in the abstinence period [27]. On the other hand, selective chemical lesions of the shell region in rats caused dissimilar responses to systemic amphetamine. Shell lesions attenuated, while core lesions augmented dose-dependent responding in a forced-choice task [31].

4.2. High line is prone to positive emotional states

The main finding of this study is that rats of the HL were highly and significantly sensitive to injections of amphetamine, while rats of the LL were not or responded marginally. Since the emission of 50 kHz vocalizations is dependent on dopamine activity and is indicative of a positive emotional state [3,10,11,16,25,26,32,33,38,40], the present result may indicate that the HL rats represent not only a stress resistant (or stress resilient) line [14] but also a line prone to positive emotional states and to higher and more prolonged effects of endogenous shell dopamine as compared to the LL rats. Based on our results and those of other studies, the rats of the HL would be expected to have higher release of endogenous shell dopamine, to be more active, stress-resistant, and readily responding to environmental challenges.
ber of FM calls resulted in the significantly higher average peak frequency of calls and significantly broader bandwidth in the HL than in the other lines. Thus, potentiation of dopamine release in the shell of the nucleus accumbens predominantly increased the FM type of calls characteristic for expression of the positive affective state in rats [16]. Consistent with this conclusion, repeated systemic application of amphetamine, which increased appetitive behaviors, was also reported to increase the number of FM 50 kHz calls but not the flat calls [1]. Conversely, impartment of functions of the mesolimbic dopamine system with injections of 6-hydroxydopamine and systemic haloperidol, selectively decreased the number of FM 50 kHz vocalizations, particularly the calls with trill components [17,18].

It remains a line of rats, which are selected on the basis of the number of emitted 50 kHz calls, are a useful model for studying brain mechanisms of different emotional phenotypes, including potentially affective personality characteristics in the mediation of drug addictions. The results indicate that the release and function of accumbens shell dopamine may be a critical factor in determining emotional phenotype of the organism. The results further indicate that the FM type of 50 kHz calls, as a useful measure of positively valenced emotional expression in rats, may serve for distinguishing and separating these phenotypes.

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