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Evaluation of Unconditioned Novelty-Seeking and *d*-Amphetamine–Conditioned Motivation in Mice

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LAVIOLA, G. AND W. ADRIANI. *Evaluation of unconditioned novelty-seeking and* d-*amphetamine–conditioned motivation in mice*. PHARMACOL BIOCHEM BEHAV **59**(4) 1011–1020, 1998.—Following repeated association between psychostimulant drugs and a distinct environment, contextual cues acquire the ability to elicit a conditioned approach response. Further, both rats and mice have a natural drive to seek for the experience of novelty, and a previously unknown environment is able to elicit an unconditioned approach response. Both the experience of novelty and amphetamine (AMPH)-conditioned effects have been associated in rodents with the activation of brain meso-limbic dopaminergic pathways. This study assessed the relative strenght of AMPH-conditioned and novelty-induced unconditioned motivations in mice. During the pretreatment period, mice were randomly assigned to three different treatment history groups, and received d-AMPH (0, 2, or 10 mg/kg IP once/day) injections for 3 days in the presence of a familiar environment. Following a 48-h washout from the last drug injection, animals were placed in the familiar and pretreatment-paired environment and challenged with either SAL (to evaluate conditioning) or a standard AMPH dose (2 mg/kg, to assess either acute drug effects or carryover influences of each animal's treatment history with the same drug). Following the opening of a partition, animals showed both a clear-cut preference for a novel environment as well as a marked novelty-induced hyperactivity. Interestingly, when mice were tested in a drug-free state in this free-choice paradigm, they expressed neither conditioning to the drug-associated environment nor carry-over effects on the novelty-induced hyperactivity profile. On the other hand, mice injected with AMPH showed a mixed profile, with AMPH 2 treatment history mice showing a conditioned preference for the familiar and drug-paired environment, whereas AMPH 10 animals preferred to spend more time in the novel compartment. Both AMPH doses were associated with an increased locomotion, whereas only the AMPH 10 dose resulted in a stereotyped behavioral syndrome, possibly reminescent of an aversive "poor welfare" condition. Thus, as a function of the drug dosage, differential positive or negative incentive properties are suggested to be evoked by the AMPH-conditioned environment. In conclusion, a reliable and useful experimental paradigm has been developed to investigate the issue of vulnerability to a variety of habit-forming agents or emotional experiences whose positive reinforcing properties may rely on a common neurobiological mechanism. © 1998 Elsevier Science Inc.

Novelty seeking Conditioning Incentive motivation Stereotypies Behavioral sensitization
d-Amphetamine Mice

ANIMALS are biologically designed to pay more attention to novel information than to a familiar one, and they actually seem to be both attracted to and activated by novel stimuli as well as by variations in the set or intensity of familiar ones (32,45). Recently, novelty seeking has been extensively elaborated as a characteristic behavioral trait, and the novelty preference paradigm has been validated in several animal models (1,2,17,25). Experimental evidence indicates that the experience of novelty is associated with the activation of the meso-limbic dopaminergic system in the CNS, because entering a novel environment in rats is associated with an elevation of dopamine levels within the nucleus accumbens (30). Furthermore, lesions of this area, induced by 6-OHDA, block the expression of novelty-seeking behavior (13,29). Indeed, experimental evidence indicates that the meso-limbic dopaminergic system in the CNS is involved in reward-related phenomena induced by salient natural stimuli as well as by drugs of abuse (15,33–35,47). In this view, the satisfaction of a novelty-stimu-

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lated "curiosity" seems to have most of the characteristics of natural rewarding events (31).

The drug-induced conditioned place preference (CPP), which is based on associative learning principles, has been widely utilized for the study of internal states of reward (10,21,22, 38,42). Cues previously paired with drug effects acquire the capacity to elicit a conditioned approach response (6), as well as to modulate the animal's physiological and locomotor parameters (3,12,40). The brain meso-limbic dopaminergic system has been shown to be involved in this reinforcement phenomenon, because the repeated injection of amphetamine (AMPH) directly into the nucleus accumbens generates a clear CPP for the drug-paired environment (7,8).

Overall, these data suggest a common neurobiological mechanism underlying both drug-induced conditioned and novelty-induced unconditioned approach responses. In both paradigms, the preference towards one of two environments has been considered as an index of an associated incentive motivation based, respectively, upon spontaneous novelty seeking and drug-conditioned reinforcing properties. Thus, it seemed interesting to evaluate the interaction between these two motivations and their relative strenght. To shed more light on the nature of these processes and underlying neurobiological mechanisms, an experimental procedure was designed, which allowed both familiarization to one specific compartment of the apparatus and repeated associations between AMPH and this environment. Animals were then tested in a free-choice novelty preference paradigm. The unconditioned novelty-related motivation towards the unknown environment was directly compared with the drug-conditioned incentive motivation for the familiar and pretreatment-paired one. This was the first aim of this study.

Locomotor hyperactivity is produced by low doses of AMPH, as well as by other drugs of abuse (19). AMPH-induced release of dopamine within the nucleus accumbens is considered to be involved in such a behavioral change (39). Because the same neural substrate seems to modulate both unobservable subjective reward and measurable locomotion, the AMPH-induced behavioral hyperactivity has been considered as an indirect index of reward (48), resembling the "euphoria" induced by this drug in humans. Interestingly, increased locomotion is expressed by rodents when forced in a novel environment (16,28). Surprisingly, no data are apparently available about locomotor effects resulting from the free-choice experience of a novel environment, which can be hypothesized to generate a profile of locomotor hyperactivity. Therefore, the second aim of the present study was to assess whether the experience of a novel environment would be associated with a hyperactivity profile.

Animals were challenged on testing day with either SAL or a standard AMPH dose, to evaluate the acute drug effects on novelty-seeking performance. Actually, the literature on this issue is mixed. It has been reported that an acute AMPH administration failed to have any effect on this parameter in rats (1), whereas an acute injection of an AMPH-related drug, such as metamphetamine, resulted in a dose-dependent impairment of novelty seeking in mice (25). Thus, it seemed interesting to extend the analysis of acute AMPH effects in this paradigm. Furthermore, because no systematic data are apparently available in the literature, the evaluation of drug effects was extended to carryover influences of each subject's treatment history with the same drug. The third aim of the present study was thus to investigate potential changes of the animal's response to natural rewarding events, such as novelty, as a function of repeated AMPH-induced stimulation.

METHOD

Subjects

One hundred and twenty male mice of the outbred CD-1 strain (Charles River, Italia), deriving from breeding pairs available in the animal colony, were used ($n = 20$ per each final group). They weighed about 32–39 g at the time of testing. Animals were housed in Plexiglas cages ($33 \times 13 \times 14$) with metal tops and a sawdust bedding. Both the animal colony and the experimental room were provided with air conditioning (temperature 21 ± 1°C, relative humidity 60 ± 10%), and with a reversed 12 L:12 D cycle (lights on at 2100 h). Water and food (Enriched Standard Diet purchased from Mucedola, Settimo Milanese, Italy) were available ad lib. All the procedures were performed in agreement with the national legislation on the care and use of laboratory animals (D. L.vo 116/92).

Apparatus

The experimental apparatus consisted of an opaque Plexiglas rectangular box with smooth walls, which was subdivided into two compartments ($20 \times 14 \times 27$). The door between the two compartments could be closed by means of a temporary partition. Two cues, one visual and one tactile, were associated with each compartment. One compartment was white and had a wide-mesh floor, whereas the other one was black and had a narrow mesh floor. Each compartment was provided with four pairs of infrared photobeams, placed on the wall at few cm from the floor, 5.5 cm apart. Each beam interruption eventually caused by mice was recorded by an IBM computer provided with a specific software. The following measures were obtained automatically: 1) time spent in each compartment, 2) activity rate in each compartment (number of beam interruptions/second), 3) frequency of passages between the two compartments (number of passages/minute), and 4) latency (time between the opening of the partition and the first entrance in the novel compartment). The whole session was automatically subdivided into 5-min intervals.

Procedure

The whole experimental schedule took a total of 6 days (see Fig. 1), each subject being tested between 1000 and 1800 h. The white compartment of the apparatus was the familiar and pretreatment-paired one. This "biased" procedure is often adopted in the literature on place conditioning [(37); for references and discussion, see (21)]. As for the case of novelty preference, the latter has been shown to be independent from environmental cues provided (1).

Day 1 (familiarization). Animals were marked on the tail, and immediately placed for 20 min in the white compartment of the apparatus.

Days 2, 3, and 4 (pretreatment period). Animals were randomly assigned to three different treatment history groups with a regimen of AMPH administration (0, 2, or 10 mg/kg, IP once/day). On each of these 3 days, animals were weighted, injected, and immediately placed in the same compartment explored during the day 1 of the schedule (familiar compartment) for a 20-min session. This was to allow an immediate association of the specific set of environmental stimuli with the onset of drug effects (21,22)

Day 5 (washout). Between the last day of pretreatment (day 4) and the testing day (day 6), an interval of 48 h was left, to avoid the influence of residual circulating levels of AMPH during the testing session.

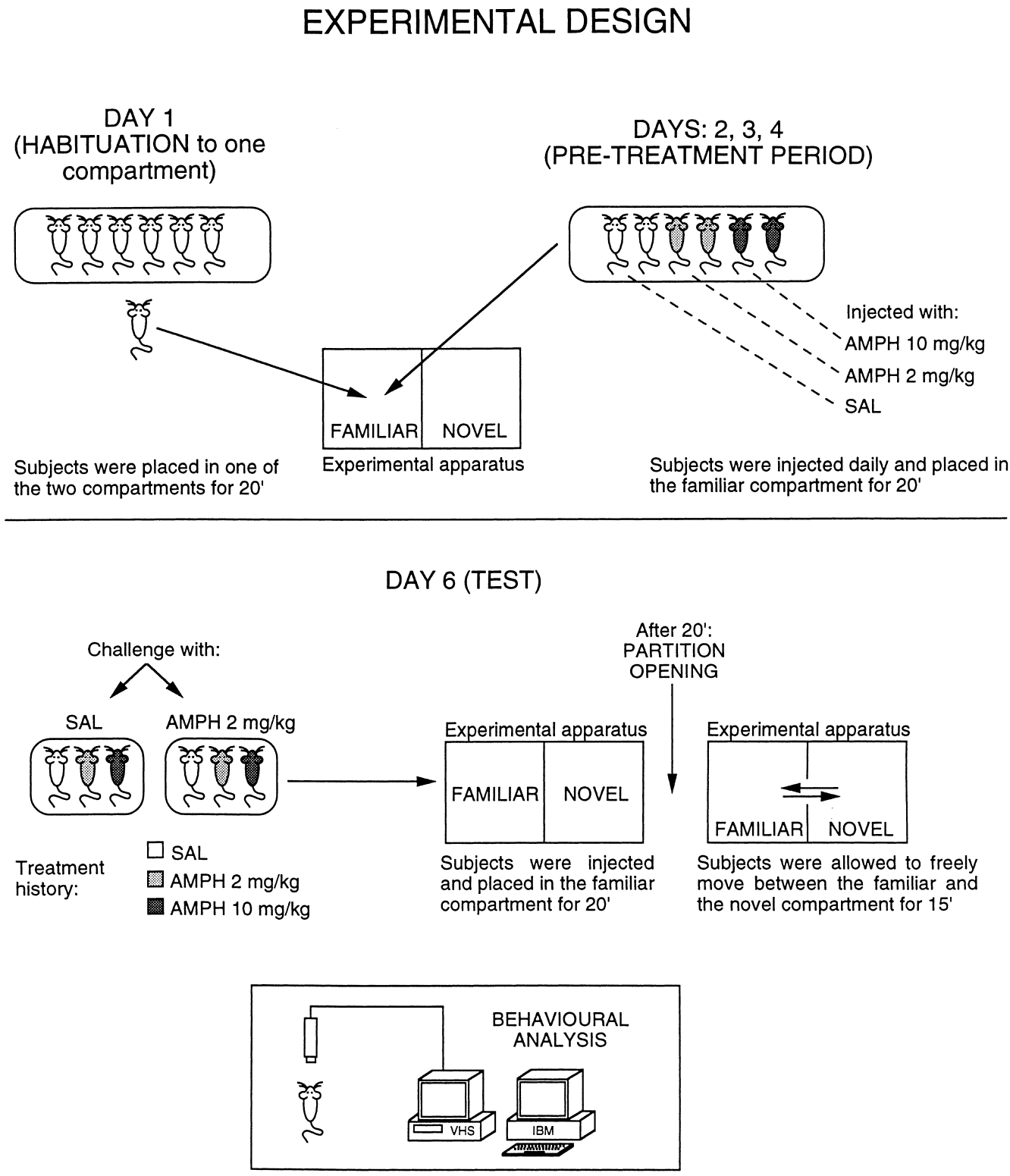

FIG. 1. Scheme of the experimental procedure.

Day 6 (test of novelty preference). Animals from each pretreatment condition were randomly assigned to a challenge with either a SAL or a standard AMPH dose (2 mg/kg). The former group was aimed to analyze possible conditioning effects, while the latter one was aimed at assessing either acute or possible carryover effects of each animal's tratment history with the same drug. Animals were injected IP and immediately placed in the familiar and pretreatment-paired compart-

ment. After 20 min, the partition separating the two compartments of the apparatus was opened, and mice were allowed to freely explore both compartments of the apparatus (the familiar and the novel one) for 15 min.

Behavioral Analysis

Suspended from the ceiling, at a distance of 100 cm over the apparatus, there was a SONY video camera connected to a JVC video recorder, which allowed video recording of the animals' behavior during the session on day 2 (pretreatment period) and on testing day. The video recording consisted of a 30-s sampling, repeated regularly every 2 min, for a total of nine samples distribuited regularly across the 20 min of observation. At the time of data analysis, the nine samples were grouped in three "intervals." The behavioral profile expressed by each animal was subsequently scored by a trained observer using an IBM computer and a specific software (THE OBSERVER v2.0 for DOS, Noldus Information Technology, Wageningen, The Netherlands). This allowed the detailed analysis of several parameters, such as latency, frequency, and duration of each behavior. The floor of each compartment was subdivided into three sections by lines placed on the video screen at the time of videotape analysis to allow the count of crossing (crossing one line with both forepaws). The following behaviors were also scored: rearing (body in vertical position), lying still (absence of any gross movement), grooming (mouth or paws on body), face washing (forepaws moving back and forth from the ears to the snout and mouth), compulsive licking (the animal licks the floor or the wall of the apparatus).

Drugs

d-Amphetamine (S.A.L.A.R.S., Como, Italy) was dissolved in a physiological solution (NaCl, 0.9%) and injected IP in a volume of 1 ml/100 g body weight. AMPH doses have been chosen in the range of those used in previous studies [for literature and discussion, see (22)].

Statistical Analysis

The general design of the experiment was a 20 litter × 3 treatment history (pretreatment dose: 0, 2, or 10 mg/kg of AMPH) × 2 challenge (testing day dose: 0 or 2 mg/kg of AMPH), as well as repeated measures on the same individual. When analyzing data from day 2 of the pretreatment period, a treatment variable was used, instead of treatment history. When analyzing data from the testing day, additional variables were specifically used, such as phase (before or after the partition opening) and compartment (familiar or novel), depending on the measure considered. When the phase variable was considered, for reasons of symmetry, only the first 15 min of the 20-min session before the partition opening were considered, and these were compared with the 15-min session following the partition opening. All data were analyzed separately by means of analysis of variance (ANOVA), using a randomized block design (11,46). Separate analyses were also performed within each challenge group, and Bonferroni correction adopted (see the Results section). Multiple comparisons within a significant interaction were performed using the Tukey HSD Test.

RESULTS

Day 2: Behavioral Analysis

A detailed behavioral analysis was performed on observational data generated on day 2 (pretreatment period) of the schedule. As expected, a dose-dependent elevation of both crossing (see Table 1) and rearing, and a corresponding reduction of time spent lying still (data not shown), were evident upon acute AMPH administration [treatment effect, $F(2, 36) = 62.32, 5.10, 9.97$, respectively, ps < 0.05 or less].

TABLE 1

MEAN (±SEM) NUMBER OF CROSSINGS AND DURATION (S) OF GROOMING, STEREOTYPED FACE WASHING, AND COMPULSIVE LICKING BEHAVIORS DURING THE FIRST DAY OF THE PRETREATMENT PERIOD (DAY 2)

	Treatment		
Behavior	SAL	AMPH 2	AMPH 10
Crossing	25.56 (±1.08)	78.78 (±3.36)*	125.10 (±4.14)*
Grooming	72.06 (±5.40)	2.91 (±0.45)*	1.59 (±0.33)*
Face washing	0.69 (±0.21)	1.20 (±0.36)	16.41 (±2.13)*
Licking	4.41 (±1.26)	1.29 (±0.66)	45.15 (±5.97)*

Subjects were injected with AMPH (treatment: 0, 2, or 10 mg/kg) immediately before being placed for 20 min in the familiar compartment. The whole 20-min session was divided in nine 30-s samples, at a regular distance of 1′30′′ from each other.

*$p < 0.01$ in multiple comparisons vs. the SAL treatment control group ($n = 40$).

For time spent grooming, measurable levels of this behavior were expressed only by SAL-injected subjects, whereas the AMPH administration completely suppressed this activity already at the lower dose [treatment effect, $F(2, 36) = 62.49$, $p < 0.001$]. On the other hand, those subjects receiving an acute AMPH 10 injection were characteristically associated with elevated portions of time spent in face washing and compulsive licking [treatment effect, $F(2, 36) = 7.07, 15.82$, respectively, ps < 0.01 or less].

Day 6: Behavioral Analysis

A behavioral scoring was also carried out on observational data from testing day 6. With respect to acute drug effects, an AMPH challenge resulted in higher levels of crossing (see Fig. 2, upper panel) and rearing (data not shown), when compared to SAL-injected controls [challenge effect, $F(1, 18) = 137.58, 10.03$, ps < 0.01 or less, respectively]. Within the SAL-injected group, animals with a treatment history of AMPH 2 or AMPH 10 expressed conditioned higher levels of crossing than the SAL-treatment history group, during the first interval of the session [treatment history × repeated measure interaction, $F(4, 72) = 4.85$, $p < 0.01$]. Conversely, within the AMPH-challenge group, animals with a treatment history of both AMPH 2 and AMPH 10 showed a dose-dependent sensitization profile for both crossing and rearing [treatment history effect, $F(2, 36) = 16.90, 5.04$, ps < 0.05 or less, respectively].

Time spent grooming (see Fig. 2, central panel) was decreased by the AMPH challenge [challenge effect, $F(1, 18) = 66.05$, $p < 0.001$]. Within the SAL-injected group, a conditioned dose-dependent reduction of grooming behavior appeared as a function of AMPH treatment history [treatment history × repeated measures interaction: $F(4, 72) = 3.49$, $p < 0.05$]. Data from the AMPH challenge group were not analyzed, because they were very close to zero.

SAL-injected animals expressed elevated levels of the stereotyped face washing behavior at the start of the session, which markedly decreased over time (see Fig. 2, lower panel). An AMPH administration produced elevated and constant

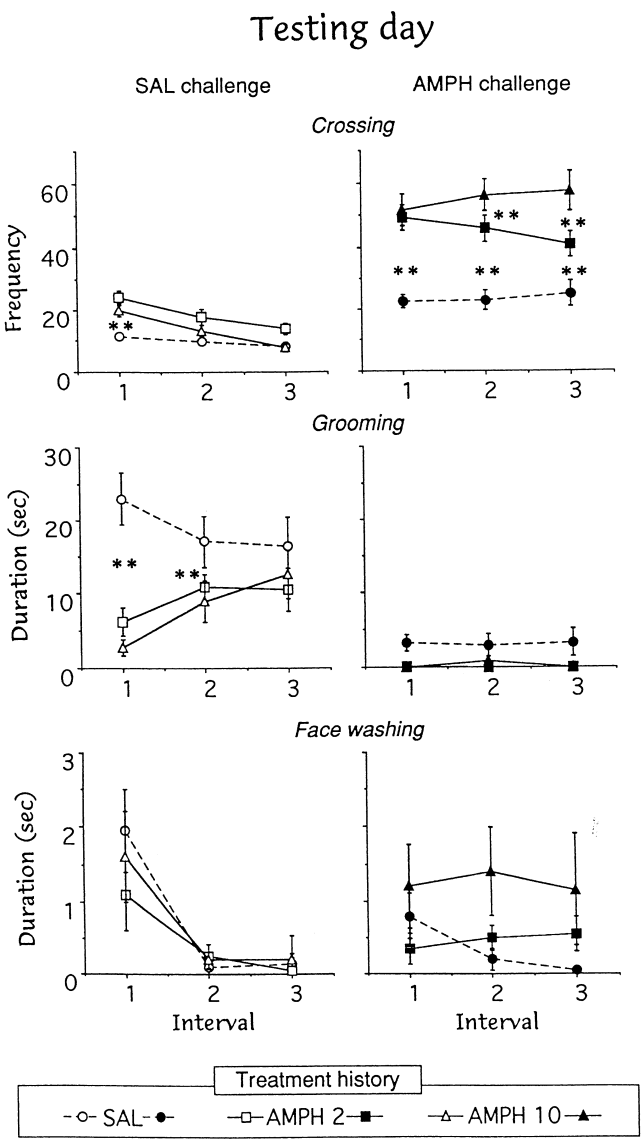

FIG. 2. Mean (±SEM) frequency of crossing and duration of grooming and face-washing behaviors, shown by subjects on testing day (day 6) before the partition opening, when challenged with either SAL (left panels) or AMPH (2 mg/kg, right panels) and placed in the familiar and pretreatment-paired compartment. The whole 20-min session was divided in three intervals. Each interval was the sum of three 30-s samples, taken at a regular distance of 1 min 30 s each other. During the pretreatment period, subjects received a daily AMPH injection (treatment history: 0, 2, or 10 mg/kg) immediately before being placed in the familiar compartment. $^{**}p < 0.01$ in multiple comparisons performed between different treatment history groups ($n = 20$).

levels during the whole session [challenge × repeated measures interaction, $F(2, 36) = 8.45$, $p < 0.01$]. Greater levels of face washing were seen in the AMPH-challenged animals that had previously received multiple injections of the higher (10 mg/kg) AMPH dose, but not the lower (2 mg/kg) one [treatment history effect, $F(2, 36) = 3.18$, $p = 0.053$]. The profile for compulsive licking (data not shown) was quite similar [treatment history by repeated measures interaction for the AMPH challenge group, $F(4, 72) = 3.62$, $p < 0.01$]. These results clearly indicate that only AMPH 10 treatment history animals developed sensitization to the AMPH-induced stereotyped component of the behavioral repertoire.

Day 6: Activity Rate

An analysis of the locomotor activity rate, expressed by animals during the novelty preference test, was performed (see the Method section). As a whole, levels of locomotion (see Fig. 3) were much higher after the partition was opened than before [phase effect, $F(1, 18) = 212.50$, $p < 0.001$]. As expected, upon an AMPH challenge, animals showed in general increased levels of activity when compared to SAL-injected controls [challenge effect, $F(1, 18) = 89.86$, $p < 0.001$].

Within the SAL challenge group (see Fig. 3, upper panel), those mice with a treatment history of AMPH 2 and AMPH 10 were significantly more active than the SAL treatment history group, during the initial 5-min interval of the phase before the partition opening [treatment history × repeated measure interaction, $F(4,72) = 3.92$, $p < 0.01$, phase before opening]. No differences due to each subject's treatment history were found in the novelty-induced hyperactivity profile. Within the AMPH challenge group (see Fig. 3, lower panel), significant differences appeared as a function of both the test phase and each animal's treatment history, $F(2, 36) = 17.96$, $p < 0.001$, also interacting with repeated measure, $F(4, 72) = 11.77$, $p < 0.001$. As expected, before the partition opening, animals showed a significant dose-dependent sensitization profile in their levels of locomotion [treatment history effect, $F(2, 36) = 23.99$, $p < 0.001$. After the partition opening, the possibility of free access to a novel environment produced a quite prominent increment of general activation in those subjects with a treatment history of SAL and of AMPH 2 (ps < 0.01). Conversely, the AMPH 10 treatment history group failed to show significant novelty-induced activation and exhibited significantly lower activity levels than those of the other two groups [treatment history × repeated measures interaction, $F(4, 72) = 10.95$, $p < 0.001$].

The influence of the compartment (familiar or novel) variable was analyzed by focusing on activity data from the phase after the partition opening (see Fig. 4). For the SAL-injected group (left panel), activity levels expressed in the novel compartment were consistently lower than those expressed in the familiar one [compartment, $F(1, 18) = 23.18$, $p < 0.001$]. No carryover effects of each subject's treatment history were found. For the AMPH challenge group (right panel), a treatment history by compartment interaction, $F(2, 36) = 7.17$, $p < 0.01$, indicated that a significant decrease in activity levels was limited to SAL and AMPH 2 treatment history groups. Interestingly, this profile was not found for AMPH 10 animals.

Day 6: Novelty Seeking

A main effect of challenge was seen on the latency to enter the novel compartment, $F(1, 18) = 27.96$, $p < 0.001$, indicating that AMPH-injected animals (47.65 ± 6.22 s) had a significantly higher latency than SAL-injected controls (17.25 ± 2.22 s). For the AMPH challenge group, the latency was also a function of each subject's treatment history, $F(2, 36) = 3.34$, $p < 0.05$, and followed a quite clear dose-dependent sensitization profile.

During the novelty preference test, carried out on testing day 6, a peculiar behavioral profile emerged following the partition opening, with mice starting to move rapidly back and forth between the two compartments of the apparatus. As expected, this profile was more marked upon an AMPH chal-

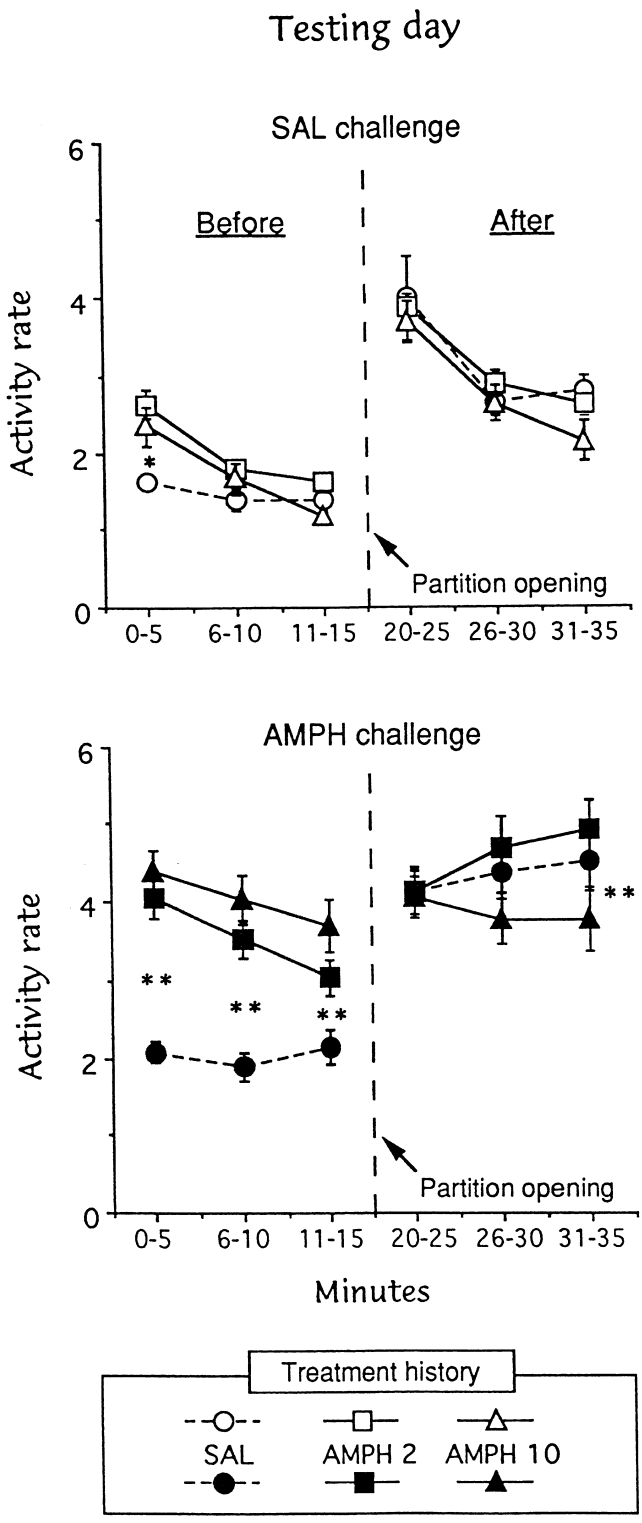

FIG. 3. Mean (±SEM) locomotor activity rate (measured as number of photobeam interruptions/s) shown by subjects on testing day (day 6), when challenged with either SAL (upper panel) or a standard AMPH dosage (2 mg/kg, lower panel) and placed in the familiar and pretreatment-paired compartment for 20 min. After this period, a partition was removed and subjects were allowed free access to a novel compartment of the apparatus for 15 min. During the pretreatment period, subjects received a daily AMPH injection (treatment history: 0, 2, or 10 mg/kg) immediately before being placed in the familiar compartment. $*p < 0.05$, $**p < 0.01$, in multiple comparisons performed between different treatment history groups ($n = 20$).

lenge [challenge × repeated measure interaction, $F(2, 36) = 12.24$, $p < 0.001$] than in SAL-injected controls. For the AMPH challenge group, the ANOVA yielded a main effect of treatment history, $F(2, 36) = 3.28$, $p < 0.05$, both animals with a treatment history with the drug [AMPH 2: 7.58 (±0.53) and AMPH 10: 5.01 (±0.62)] showing a significant reduction in the frequency of passages/min between the two compartments, when compared to those from the SAL [9.03 (±0.78)] treatment history group.

Regarding the percentage of time spent in the novel compartment (see Fig. 5), the ANOVA yielded a significant challenge by repeated measures interaction, $F(2, 36) = 19.38$, $p < 0.001$. In particular, SAL-injected controls, which were higher immediately after the partition opening, then showed a classical habituation profile throughout the session (left panel), whereas AMPH-injected mice showed a quite opposite profile (right panel). Thus, the peak of preference for the novel compartment resulted to be somewhat delayed upon an AMPH challenge. In this latter group, a mixed profile of effects was also found during the session, as a function of each subject's treatment history with the same drug, $F(4, 72) = 5.83$, $p < 0.001$. Indeed, mice from the AMPH 2 treatment history group spent a reduced portion of time in the novel compartment when compared to SAL treatment history controls. Conversely, AMPH 10 treatment history subjects were associated with increasing levels of novelty preference, as the session progressed.

DISCUSSION

The results of the present study can be briefly summarized as follows: 1) as a whole, levels of locomotion were much higher after the partition was opened than before. Interestingly, within the SAL-injected group, the amount of activity showed in the novel compartment was consistently lower than that expressed in the familiar one. No carryover effects of each subject's treatment history were found to affect the novelty-induced hyperactivity profile. Within the AMPH challenge group, before the partition opening, animals showed a significant dose-dependent sensitization profile in their levels of locomotion. After the partition opening, the possibility of free access to a novel environment produced a further prominent increment of general activation in those subjects with a treatment history of SAL and AMPH 2, but not of AMPH 10. Interestingly, SAL and AMPH 2 treatment history groups, but not AMPH 10 animals, showed reduced activity levels in the novel compartment, when compared to the familiar one. 2) SAL-injected controls spent a higher percentage of time in the novel compartment than in the familiar one, and no carryover effects of treatment history were evident. Within the AMPH challenge group, mice with an AMPH 2 treatment history spent a reduced portion of time in the novel compartment when compared to SAL treatment history controls. Conversely, AMPH 10 treatment history subjects were associated with increasing levels of novelty preference, as the session progressed.

Results from the present study confirm and extend previous observations [(1,25); see also the introductory paragraphs]. When assessed in a free-choice paradigm, animals expressed a

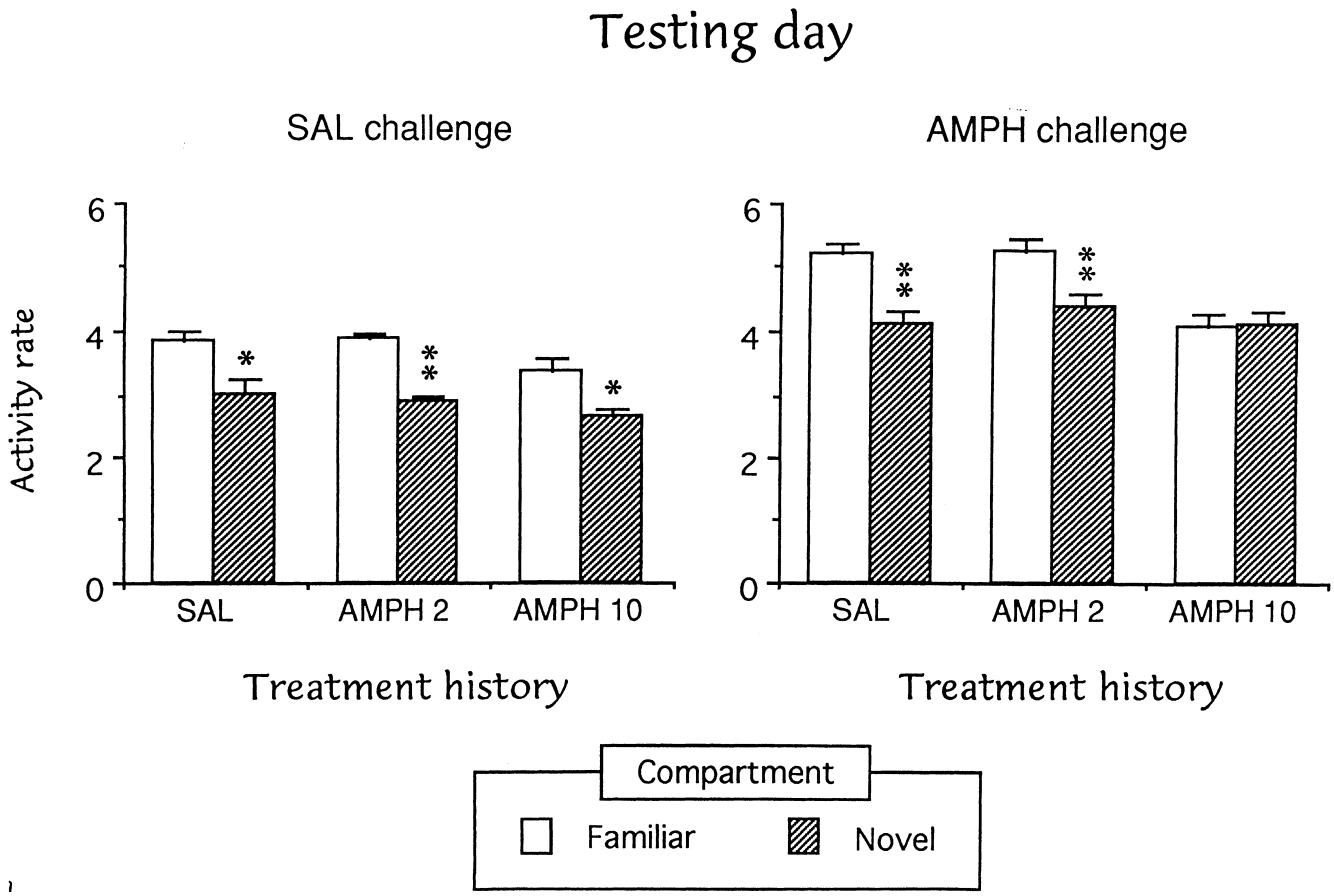

FIG. 4. Mean (±SEM) locomotor activity rate shown after the partition opening by subjects on testing day (day 6), either in the novel or in the familiar and pretreatment-paired compartment. Animals were challenged with either SAL (left panel) or AMPH (2 mg/kg, right panel). Subjects are the same as in Fig. 3. *$p < 0.05$, **$p < 0.01$, in multiple comparisons performed between the two compartments ($n = 20$).

clear-cut preference for novelty. This approach response has been considered as an indirect behavioral evidence of an internal state of reward, because it is unconditionedly elicited by a number of rewarding natural stimuli (14).

The discovery of a novel environment and the possibility of free access to it came in association with a prominent increment of general activation, clearly indicating that the experience of novelty had an arousing effect in rodents. Furthermore, levels of locomotor activity expressed during the exploration of the novel compartment were always and consistently lower than those expressed in the familiar one, and this was probably a byside effect of the concomitant assessment of potential danger in a completely unknown environment. Consistently, it has been reported (5) that, during exploration of a novel environment, mice express both approach (sniffing and smelling) and avoidance (stretched attend, stretched advance) reactions. This behavioral pattern has been considered as an index of risk assessment, because it is markedly expressed when mice are in the presence of the odor of a predator (4). Thus, we showed evidence that two kinds of phenomena were elicited by the experience of novelty, namely an increased general arousal and a slight behavioral inhibition. The former has been suggested to be an index of reward (see the introductory paragraphs), whereas the latter perhaps seems to come in association with a certain degree of anxiety.

As a whole, after the partition opening on testing day 6, animals injected with SAL showed a novelty-induced hyperactivity profile, but did not show carryover effects of each subject's treatment history. This suggests that the neurobiological pathways responsible for the novelty-induced hyperlocomotion were not sensitized by a repeated AMPH administration. Conversely, within the AMPH challenge group, a clear-cut and dose-dependent sensitization profile emerged, the higher AMPH dose inducing the greater sensitization. Therefore, in apparent contrast with data reported in the introductory paragraphs, a dissociation seems to emerge between novelty- and AMPH-induced hyperlocomotion. After the partition opening, however, activity levels exhibited by animals with both SAL and AMPH 2 treatment history were further increased. This can be interpreted as being the result of the experience of novelty. The latter, in fact, might have produced an additional and unconditioned increment in dopamine release within the same brain area [e.g., the nucleus accumbens; see (18,30,36). Hence, sensitization to amphetamine and the experience of novelty resulted in additional locomotor stimulation. As it was hypothesized in the introductory paragraphs, this may be due to the activation of partially overlapping neural substrates.

It should be noted that, upon an AMPH challenge, a very peculiar behavioral profile was found for those subjects with a treatment history of AMPH 10, compared with the other two

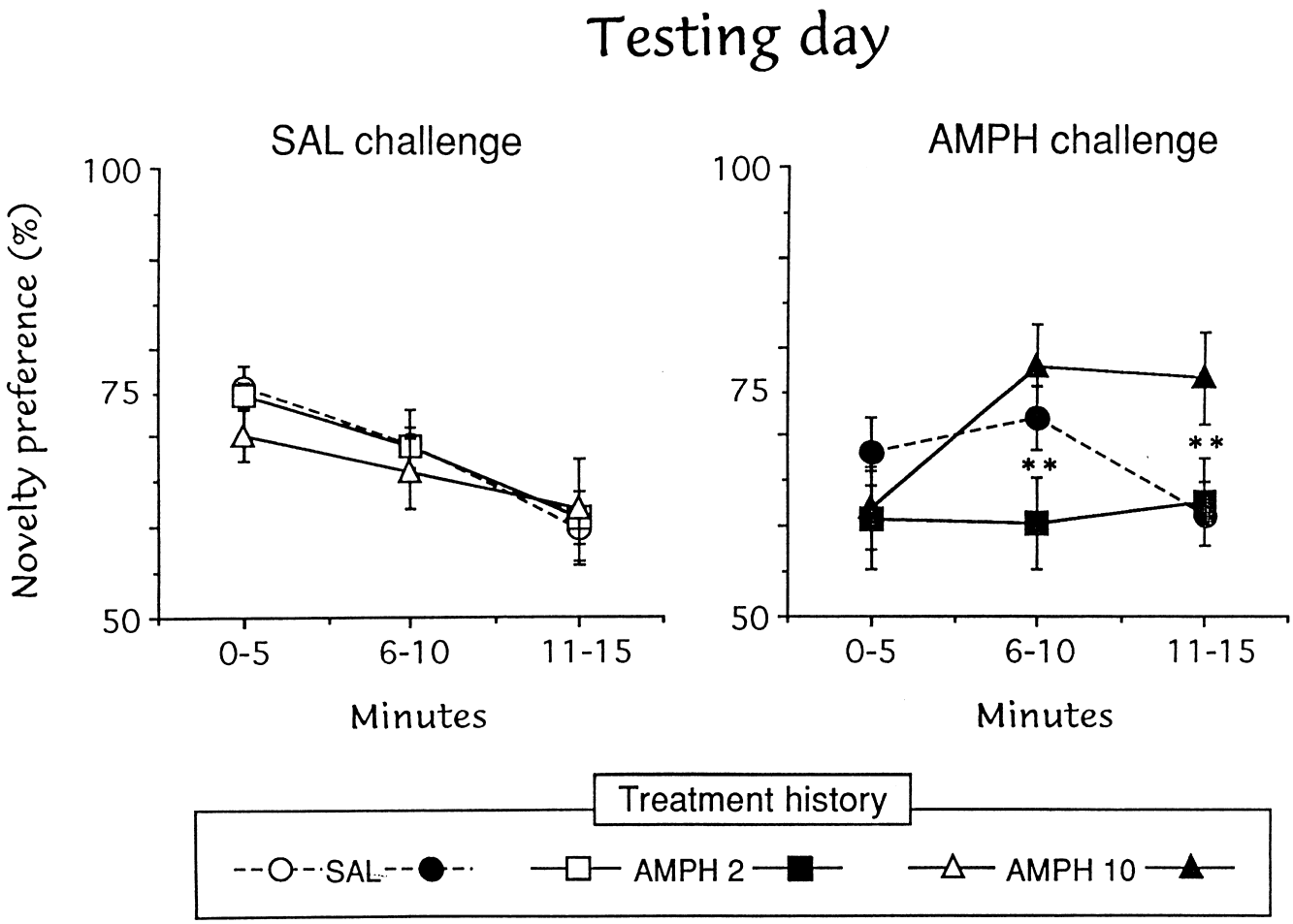

FIG. 5. Mean (±SEM) percentage of time spent in the novel compartment by subjects on testing day (day 6), when challenged with either SAL (left panel) or AMPH (2 mg/kg, right panel). After a 20-min period spent in the familiar and pretreatment-paired compartment, a partition was removed and subjects were allowed free access to a novel compartment of the apparatus for 15 min. During the pretreatment period, subjects received a daily AMPH injection (treatment history: 0, 2, or 10 mg/kg) immediately before being placed in the familiar compartment. $^{**}p < 0.01$, in multiple comparisons performed between different treatment history groups ($n = 20$).

groups (see Figs. 3 and 4). It can be hypothesized that extremely elevated (perhaps maximal) activity levels, produced by sensitization, prevented the possibility of a further novelty-induced increment of locomotion in this group.

Acute AMPH administration was responsible for a dose-dependent increase of the latency to enter the novel environment. Thus, a shift to the right in the peak of novelty preference appeared. Such a result is not in agreement with the available literature, which is mixed. No direct comparisons can be made, however, because of relevant differencies in the experimental paradigms used [(1,25); see also the introductory paragraphs].

In the present study, to assess the development of a drug-induced conditioning as well as sensitization to the behavioral drug effects, the familiar compartment of the apparatus was associated with three different levels of AMPH administration. Interestingly, when considering the time spent in the novel compartment, conditioned carryover effects, depending on each subject's treatment history, were not found in those animals assessed in a drug-free state (testing day). This profile might be the result of a balance between two contrasting drives, namely 1) the expected conditioned preference for the drug-associated environment, and 2) the unconditioned novelty seeking. Such a result seems in agreement with previous studies [(9,41); see also (43)], in which the novelty-induced motivation was able to interfere with the estabilishment of a fully fledged conditioned place preference. An alternative explanation of the present results can also be based on an extinction phenomenon, which might have been produced by exposing the animal in drug-free state to the familiar and pretreatment-paired environment (phase before the partition opening).

Conversely, we showed evidence that a carry-over influence of each animal's treatment history can be revealed upon an AMPH-induced stimulation. In fact, mice from the AMPH 2 treatment history group spent less time in the novel compartment than those with a SAL treatment history (see Fig. 5). This is suggestive of a drug-induced conditioned place preference for the familiar and AMPH 2 pretreatment-paired compartment, which contrasted the natural drive to approach the novel environment. Thus, at least for the AMPH 2 treatment history group, the two compartments of the apparatus resulted to be associated to conditioned and unconditioned incentives, respectively.

Again, in clear contrast with the other groups, those subjects that underwent a repeated administration of a very high drug dosage (AMPH 10) during the pretreatment period showed a prominent increment of time spent in the novel environment during the session. It can be hypothesized that drug-related negative reinforcing properties became associated with the familiar and pretreatment-paired compartment (22–24,44). Similarly, in a novelty-seeking experiment in which novel objects were added either to the novel or to the familiar environment, positive or negative contribution to the novelty-induced place preference could be produced (26). Thus, it can be hypothesized that the AMPH 10 pretreated subjects of the present experiment developed a conditioned aversion for the familiar and pretreatment-paired environment, and, when given the possibility of a free choice, they took refuge in the other one. Consistently, these animals did not show the "risk assessment-related" reduction in activity levels, which otherwise characterized the profile of the other two treatment history groups in the novel compartment (see above and Fig. 4).

In response to AMPH administration, rodents generally show either a profile of locomotor hyperactivity or a stereotyped behavioral syndrome, as a function of drug dosage [see, e.g., (19,22,39). Behavioral sterotypies have been proposed to serve as a coping mechanism for drug-induced excessive arousal (20,27). Because of such a "poor welfare" experience, stereotypies might also underlie potential AMPH-related aversive properties (22–24,44). Results derived from the detailed behavioral analysis carried out in the present study seems to be consistent with this hypothesis. In fact, on day 2 of the pretreatment period, only those subjects receiving an acute AMPH 10 injection were associated with elevated levels of stereotyped face washing and compulsive licking behaviors. When challenged with AMPH on testing day 6, only AMPH 10 treatment history mice expressed increased levels of stereotypies, whereas animals with an AMPH 2 treatment history showed sensitization only to the locomotor component of the behavioral repertoire and did not exhibit drug-induced stereotypies.

In conclusion, the seeking for novelty has been shown to have much of the characteristics of a strong unconditioned drive, in that the approach response, which allows an experimental animal to discover and explore an unknown environment, also came in association with a marked arousal. The experimental paradigm adopted in the present study allowed an evaluation of the nature and relative strength of two different motivations, namely unconditioned novelty-seeking and drug-induced place conditioning (see also the introductory paragraphs). We provided evidence that the latter can be masked by the former when animals are tested in a drug-free state, whereas the presence of both drug-related positive and negative reinforcing properties—as a function of drug dosage—can be revealed upon an AMPH-induced stimulation. Interestingly, the present results also suggest that the neurobiological pathways responsible for the novelty-induced hyperlocomotion are not sensitized by a repeated AMPH administration.

Thus, a reliable and useful model has been developed to investigate the issue of vulnerability to a variety of habit-forming agents or emotional experiences whose positive reinforcing properties may rely on a common neurobiological mechanism.

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