3.2. Drug-primed reinstatement 7
3.2.1. Weak effects of drug priming on reinstatement behavior 7
3.2.2. Transient drug-primed reinstatement behavior 7
3.3. Cue-induced reinstatement 7
3.3.1. Strong effects of drug-associated cues on reinstatement behavior 7
3.3.2. Long-lasting cue-induced reinstatement behavior 7
3.4. Stress-stimulated reinstatement 7
3.5. Correlation between drug SA, extinction, and triggering factors of reinstatement behavior 8
4. Concluding remarks 8
Acknowledgements 8
References 9

1. Introduction

A major clinical problem in treating drug abusers or addicts is the high rate of relapse to abuse even long after abstinence [40,41,63,96]. **To address this issue, much effort** has been made in the establishment of extinction-reinstatement procedures in experimental animals [10,18,19,30,72,80], which are currently used to mimic some aspects of drug relapse in human addicts. There are at least two typical versions of extinction-reinstatement paradigms: the drug self-administration (SA) and extinction-reinstatement procedure, and the drug-conditioned place preference and extinction-reactivation procedure. The drug SA version of the extinction-reinstatement procedure is based on the findings of similarities in the development of drug dependence/addiction between humans and laboratory animals (Table 1), including intravenous drug SA behavior (e.g. [7]), escalated drug consumption [1,52], compulsive drug-taking behavior [15,20,92], cue-induced reinstatement [23,25,29,39,60,64,94,97], drug-primed reinstatement [19,30,39,80,99], and stress-triggered reinstatement [4,27,80]. The relevance of these extinction-reinstatement behaviors to human dependence/addiction has been reviewed and discussed extensively elsewhere [26,43,72,73]. Using this procedure, potential anatomical neuronal substrates and neurotransmitter systems during the development of drug **dependence/addiction have been i**dentified [28,71,80,94], and clinical studies support these findings [5,35–37,45]. Clinical therapeutic candidates have also been tested using the extinction-reinstatement procedure in experimental animals [73,90].

Epidemiological studies suggest that genetic factors contribute to 30–60% of the variability in drug dependence/addiction and alcoholism [44,47,62,89,91,93]. Through the over-expression or inactivation of target genes via viral vectors (e.g. over-expression vector or siRNA vector) or antisense oligonucleotides, the SA–extinction-reinstatement procedure in rats has been used to investigate genetic factors involved in drug dependence/addiction including drug relapse [62]. To date, however, the genetically modified laboratory animals available have mainly been inbred mouse strains. Therefore, it is essential for researchers to extend the drug SA–extinction-reinstatement procedure in rats to mice. Since intravenous drug SA behavior was reported in mice by Carney et al. [7] **in 1991, several specific genes/proteins** have been identified as neural substrates involved in the early stage of drug dependence/addiction, including an acetylcholine receptor containing beta 2, the serotonin 1B receptor, the dopamine D2 receptor, the mu-opioid receptor, metabotropic glutamate receptor 5, cannabinoid receptor 1, the Kir3 potassium channel subunit, **homer 2, and the tissue plasminogen activator** [50,61,77,83,98]. In addition, several lines of evidence have indicated that there is considerable individual variability in the propensity for drug **relapse in human addicts or laboratory animals** [4,20,47,51,93]. Although susceptibility to drug relapse might be, at least in part, due to genetic factors [4,17,67,78], it largely remains to be determined which specific genes are involved in the propensity for drug relapse. Since the year 2002, researchers have managed to develop a drug SA–extinction-reinstatement procedure in mice [29,39,48,88,97,99]. Using this procedure, it has been shown that specific genes are associated with vulnerability to the reinstatement of drug-seeking behavior [59,69,100,101]. Based on recent documented information and our own experiences, in this review, we firstly describe some procedural considerations for the successful establishment of an SA–extinction-reinstatement procedure in mice, and then summarize some behavioral characteristics of mouse strains under the SA–extinction-reinstatement procedure.

Table 1
Similarities in drug dependence/addiction between humans and experimental animals

Operant behavioral items	Humans	Non-human primates	Rates	Mice
Drug self-administration	○	○	○	○
Escalated drug consumption	○	○	○	N.D.
Compulsive drug-taking	○	○	○	N.D.
Relapse				
Drug-associated cues	○	○	○	○
Drug priming (i.p.)	○	○	○	Transient
Stress stimulation	○	○	○	Limited
Specific candidate genes	?	?	?	?

○ indicates similar; ? indicates unclear; N.D. indicated not determined.

2. Procedural considerations

The procedural considerations for SA–extinction-reinstatement in rats have been well described elsewhere [73]. The practical considerations for surgery to implant the catheter and drug SA training in mice have also been discussed in detail elsewhere [85]. To date, few procedural considerations or suggestions have been available for drug SA–extinction-reinstatement in mice [78]. In this section, attention will be paid to a number of methodological considerations in extinction training and subsequent tests for drug-primed, cue-induced, and stress-triggered reinstatement behavior in mice. Also, whether certain procedural factors in drug SA training affect extinction and subsequent tests for reinstatement of extinguished drug-seeking behavior in mice will be discussed.

2.1. Extinction

In an SA–extinction-reinstatement procedure, extinction refers to a progressive decrease in drug-associated operant responding when the drug is no longer available [62]. The extinction training is usually introduced prior to reinstatement tests when the animals acquired stable drug SA behavior.

2.1.1. *Conditions for extinction* training

Two sets of conditions have been used for extinction training in animals: extinction training (set# 1) with neither drug-conditioned cues nor availability of a drug solution versus extinction training (set #2) with drug-conditioned cues but without the infusion of a drug solution. To determine the cue-induced reinstatement of extinguished drug-seeking behavior, set# 1 of conditions should be employed for extinction training following the acquisition of stable drug SA behavior [29,39,48,59,69,97,99,100]. Both sets of extinction conditions could be used to determine the drug-primed or stress-triggered reinstatement of extinguished drug-seeking behavior. For example, a few laboratories have demonstrated that after extinction training under set #1 of extinction conditions, a priming injection of drug alone reliably reinstates drug-seeking behavior in monkeys or rats without the presentation of cocaine-paired stimuli (e.g. [2,52,79]). In most published drug-primed reinstatement studies, however, drug-associated cues are presented either contingently and/or non-contingently during both extinction training and drug-primed reinstatement testing, namely extinction training preformed under set #2 conditions (e.g. [4,29,39,97,99,100]). One reason for this is that repeated presentations of previously drug-associated cues during the testing period seem to facilitate and prolong effects of the one-shot priming injection of a drug or transient stress stimulation on subsequent reinstatement behavior. For example, cocaine-paired external stimuli have been reported to enhance the reinstating effectiveness of non-contingent cocaine administration in rats and monkeys previously trained under second-order schedules of drug reinforcement [42,79]. Similarly, it has been reported that the combination of i.p. cocaine + contingent cocaine-paired external stimuli significantly reinstated responding, whereas the i.p. priming injection of cocaine alone did not [75]. It has also been reported in cocaine-trained rats that the non-contingent presentation of drug-associated external stimuli in combination with a priming injection of d-amphetamine produced greater reinstatement behavior and enhancement of dopamine efflux in the nucleus accumbens than amphetamine alone or non-contingent stimuli alone [22]. Although the reinstating power of these stimuli is significantly attenuated by repeated presentation during extinction, it is possible that they still possess some reinstating efficacy. The absence of drug-paired external stimuli, in any form, in the testing for reinstatement induced by a drug alone might play an important role in reducing the magnitude of the drug-primed reinstatement effect to insignificant levels [52]. Therefore, it has been postulated that the absence of contingent drug-associated cues during extinction and subsequent reinstatement testing decreases the effectiveness of a non-contingent drug alone as a reinstating stimulus [52,99]. Interestingly, it has been shown in rats that drug-primed or stress-triggered reinstatement behavior is critically dependent on repeated presentations of the contingent drug-associated cues [52,74,75].

Under set #1 of extinction conditions, the absence of drug-contingent cues will reduce the number of extinction training sessions (days) needed to achieve similar extinction criteria (e.g. 6–10 daily sessions of training in rats). Given that drug-primed reinstatement is transient [99], the decreased number of extinction training sessions needed to achieve the criterion may be critical for successful observation of the drug-primed reinstatement behavior in mice. Under set #2 of extinction conditions, however, it may take much longer for animals, especially for mice, to achieve the extinction criteria. For example, it has been reported in rats that drug-related cues induce an enduring resistance to extinction of cocaine-seeking behavior [95]. For mice, we should weigh the duration of extinction training and the facilitation of drug-contingent cues for drug-primed or stress-triggered reinstatement behavior because the drug-primed or stress-triggered reinstatement behavior in mice is transient or limited [4,29,39,48,97,99]. Thus, the careful selection of extinction conditions will be important to the successful establishment of a SA–extinction-reinstatement procedure in mice.

2.1.2. Criteria for extinction training

There are at least three types of criteria for extinction training in mice: absolute criteria (# 1), where the number of active operant responses (nose-poke or lever-press) is less than or equal to the absolute number of active operant response (e.g. ≤20) in two or three consecutive sessions [29,39,48,59,99]; relative criteria (#2), where the number of active operant responses (nose-poke or lever-press) is less than or equal to the relative percentage (e.g. 25–30%) of previous baseline operant responses in each session of drug SA during a stable phase [69]; and composite criteria (#3), where the number of active operant responses (nose-poke or lever-press) meets any of the absolute or relative criteria (e.g. [97]).

It has been observed that mice exhibit many more active operant responses to seek the infusion of a drug solution during the first session of extinction training than do rats [78,97,99,100]. Furthermore, mice seem to be much more resistant to extinction training as compared with rats [29,78,97,99,100]. To shorten the duration of extinction training in mice, in some cases, relative or composite criteria may be helpful to successfully observe drug-primed or stress-triggered reinstatement of extinguished drug-seeking behavior [78,97].

2.1.3. Potential effects of several factors during drug SA on subsequent extinction training

2.1.3.1. Schedules of reinforcement. Drug SA can be performed under a single FR, multiple FR, or progressive ratio (PR) schedule of reinforcement [21,29,39,84,87,97,99,100]. It has been postulated in mice that drug SA under the PR schedule will give rise to unnecessary difficulty in the subsequent extinction training, since mice show stronger resistance to extinction training [29,78,97]. Furthermore, drug SA training under the PR schedule of reinforcement may not only extend the duration of subsequent extinction training, but also facilitate cue-induced reinstatement of extinguished drug-seeking behavior in rodents. Similarly, drug SA training under a higher FR schedule of reinforcement may also have some impact on subsequent extinction training and cue-induced reinstatement behavior. Importantly, animals are usually subjected to extinction training under the same schedule as that used for the stable phase of drug SA training. Relative to the PR schedule of reinforcement, the FR schedule would be better for drug SA training when targeting drug relapse in mice. In our experience, drug SA training under the FR1/2 schedule of reinforcement seems to be useful for shortening the duration of the subsequent extinction phase [97,99]. After about 10 daily 3-h sessions of extinction training, mice could meet the extinction criterion (less than 25 active nose-poke responses or 30% of active nose-poke responses during the stable self-administration phase in two consecutive daily sessions) [97,99]. In contrast, it has been reported that mice were considerably resistant to extinction training (18.3 ± 2.7 days) to meet a similar criterion (less than 25 active lever-pressing responses in two consecutive daily 2-h sessions) [29]. Here, we recommend a combination of the FR1 and FR2 schedules (FR1/2 schedule) for drug SA training and FR2 for extinction and subsequent tests for reinstatement behavior in mice [97,99,100]. In our studies, once the mice could make a minimum of 60% nose-poke responses in the active hole and received no less than 10 infusions of addictive drug solution over two consecutive sessions under an FR1 schedule, the drug reinforcement program shifted to an FR2 schedule [97,99].

2.1.3.2. Session time. It has been reported that session time for drug SA plays an important role in the subsequent extinction responding and drug-primed reinstatement behavior in rodents [52,55]. Although there is wide range of session times for drug SA training in rodents, from 30 min to 24 h [4,7,29,39,69,97–100], sessions of 30 min to 3 h have been mostly used for the SA–extinction-reinstatement procedure in mice, and the session time for extinction and subsequent tests for reinstatement behavior usually is the same as that used for prior drug SA training [4,29,48,69,97,99,100]. To target specific genetic factors involved in extinction responding or incubation of drug-craving behavior, however, researchers have to take a longer session time (e.g. a daily 6-h session) into consideration [52,55,56].

2.1.3.3. Operant training and food/water restriction prior to or during drug SA training. To accelerate the acquisition of drug SA behavior in rodents, it has become popular for researchers to employ operant training with natural rewards (such as food, water, sucrose, etc.) prior to catheterization for drug SA and food/water restriction during the drug SA training. However, the operant training with natural rewards or food/water restriction has been reported to accelerate the acquisition of stable drug SA behavior [4,9,11,29,59,69], increase resistance to extinction of cocaine-taking behavior [16], and facilitate reinstatement of extinguished cocaine-seeking behavior in rodents [8,39]. If possible, researchers should use neither operant training prior to drug SA nor food/water restriction during drug SA to avoid confounding effects in extinction-reinstatement studies. For some addictive reinforces such as morphine, nicotine, and alcohol, however, operant training with natural rewards and/or food/water restriction seems to be necessary [4,69,88,98,101]. In this case, researchers have to consider the profound impact of prior operant training and/or food/water restriction in their experimental design and the interpretation of the SA–extinction-reinstatement studies in mice. Notably, when the same experimental chamber is used for food-reinforced operant training and subsequent drug SA training, the resumption of operant responding during the tests for drug reinstatement behavior may be due to food extinction responding or reinstatement of food-seeking behavior rather than drug-seeking behavior. If this is the case, researchers have to design additional experiments to clarify the confounding influence of prior operant training and/or food/water deprivation on extinction and subsequent tests for reinstatement of drug-seeking behavior. For example, researchers can examine reinstatement of food-seeking behavior in a separate group of animals that are subjected to similar operant training with natural rewards and/or food/water deprivation [100].

2.2. Drug-primed reinstatement behavior

In an SA–extinction-reinstatement procedure, reinstatement refers to the resumption of extinguished drug-seeking behavior following exposure to addictive drugs, drug-associated cues, or stress stimulation [73]. Accordingly, there are three subtypes of tests for reinstatement behavior: non-contingent drug-primed (typically i.p.), cue-induced, and stress-stimulated reinstatement of extinguished drug-seeking behavior. Drug-primed reinstatement refers to the resumption of extinguished drug-seeking behavior following exposure to non-contingently priming injections of addictive drugs (i.p., i.v. or s.c.).

2.2.1. "Between-session", "between-within-session", and "within-session" schedules

According to the timeline, the tests for reinstatement of extinguished drug-seeking behavior could be classified into "between-session", "between-within-session", and "within-session" schedules [73]. The "between-session" schedule is that in which drug SA training, extinction training, and subsequent tests for reinstatement behavior are conducted on separate days [18]. The "within-session" schedule is that in which drug SA training, extinction training, and tests for reinstatement behavior are conducted on the same day [86]. The "between-within-session" schedule is that in which drug SA training is conducted over days, but the subsequent extinction training and tests for reinstatement behavior are determined on the same day following different periods of drug withdrawal [19].

For an SA–extinction-reinstatement procedure in mice, "between-session" [29,48,97,99,100] and "between-within-session" [39] schedules have been used to determine drug-primed reinstatement of extinguished drug-seeking behavior. In the "between-session" schedule, longer extinction periods increase the similarity of the animal model to drug relapse in the human setting [10]. However, repeated testing under extinction conditions may result in the attenuation of operant responding to drug-priming injections according to the "between-session" schedule,. Using the "between-within-session" reinstatement schedule, Highfield et al. [39] have found that 129X1/SvJ mice show modest drug-primed reinstatement behavior after a priming injection with cocaine at a dose of 6.0 mg/kg (i.v.), although lower doses of cocaine (1.5 and 3.0 mg/kg, i.v.) failed to reinstate cocaine-seeking behavior. In contrast, using the "between-session" reinstatement schedule, Fuchs et al. [29] have found that in C57BL/6 mice, cocaine-seeking behavior is not reinstated after a priming injection with a wide range of doses of cocaine (0, 1, 2.5, 5, 10, 20, and 40 mg/kg, i.p.) according to a between-subjects design. We have previously reported that the drug-primed reinstatement behavior in mice seems to be transient [99]. If this is the case, it would seem difficult to observe reinstatement behavior induced by the priming injections of addictive drugs, after a prolonged period of repeated extinction training in the "between-sessions" schedule. Thus, the different time schedules used by the two research groups may provide one possible explanation for this discrepancy (the "between-within-session" vs. the "between-session"). The i.v. priming injections of addictive drugs seem to be much more effective of reinstating extinguished drug-seeking behavior than i.p. priming injections in mice [39,48,97]. However, the i.v. drug-primed reinstatement behavior following longer extinction periods according to the "between-within-session" schedule seem not to be feasible, since catheter patency is shorter in mice than in rats. In addition, the "between-within-session" or "within-session" schedule appears to be a better choice for drug-primed reinstatement behavior in mice, especially when the screening of therapeutics is the major goal, because the extinction training and subsequent test for drug-primed reinstatement behavior occur on the same day.

2.2.2. Between-subjects versus within-subjects design

Drug-primed reinstatement behavior can be determined with a between-subjects or within-subjects design. The advantages of the between-subjects design are as follows. First, the dose-dependent response tests for drug-primed reinstatement behavior can be determined on the same day and under the same conditions. Second, the effective priming dose of addictive drugs to reinstate drug-seeking behavior can be clearly shown in the dose-dependent response curve for drug-primed reinstatement behavior. However, this design requires the use of a large number of animals, and a lot of effort for prior drug SA training, especially in the case of mice. To date, a between-subjects design has mostly been used to test drug-primed reinstatement behavior in rodents [29,39]. To save on the number of animals used in experiments, however, a within-subjects design has also been employed to test

drug-primed and cue-induced reinstatement behavior [48,99,100]. Although it has been shown that i.p. priming injections of addictive drugs reliably reinstate extinguished drug-seeking behavior in rats using a between-subjects design, the i.p. priming injection of cocaine with a wide range of doses (6–40 mg/kg) failed to reinstate extinguished cocaine-seeking behavior in C57BL/6J and 129 mice [29,39]. Using a within-subjects design, however, it has been recently shown that the i.p. priming injection of methamphetamine (METH) dose-dependently reinstated drug-seeking behavior in the C57BL/6 strain, and subject-regulated i.v. injection of cocaine reliably induces reinstatement of drug-seeking behavior in the C56BL/6Jstrain [29,99]. **One may argue that under a within-s**ubjects design, repeated testing over several consecutive daily sessions in the same group of animals might reduce the effectiveness of the drug alone as a reinstating stimulus. Instead, researchers should consider the opposite case, in which once triggered by higher effective doses of addictive drugs, the drug-seeking behavior may remain until, even beyond, the next daily test. Actually, previous reports have confirmed that repeated testing does not play a role in the lack of effects of non-contingent drugs alone [29,99].

It would be interesting to further investigate whether a within-subjects design can overcome the weak effects of i.p. priming injections of other addictive drugs on the reinstatement of extinguished drug-seeking behavior in mice.

2.2.3. Priming by intraperitoneal (i.p.) injection versus intravenous (i.v.) infusion

Both i.p. and i.v. priming injections of addictive drugs have been used to test drug-primed reinstatement behavior in rodents [29,39,48,52,72,99,100]. In rats, i.p. priming is widely used and consistently effective of reinstating extinguished drug-seeking behavior [72]. In mice, however, its effectiveness in reinstating extinguished drug-seeking behavior has been inconsistent [29,39,48,97,99]. For example, using a between-subjects design, the i.p. priming injection of cocaine at a wide range of doses (1.0–40.0 mg/kg) failed to reinstate drug-seeking behavior in the C57BL/6 or 129X1/SvJ mouse strain [29,39,85]. Also, we have previously observed that using a between-subjects design, the i.p. priming injection of METH at doses of 0.5 and 1.0 mg/kg failed to induce drug-seeking behavior in the C57BL/6 strain [97], although similar doses reliably provoke the reinstatement **of extinguished METH-seeking behavior in** rats [2]. In contrast, using a within-subjects design, the i.v. priming infusion of cocaine seems to be effective at reinstating extinguished cocaine-seeking behavior in C57BL/6 or 129X1/SvJ mice, although one study employed a "between-within-session" schedule [39], and the other, a "between-session" schedule and a subject-regulated priming infusion of cocaine [48]. Similarly, we have reported that at the doses examined, a combination of an i.v. non-contingent priming injection and self-infusions of METH reinstated drug-seeking behavior in the C57BL/6Jstrain [97].

Although the potential mechanism(s) underlying the difference in reinstatement of extinguished drug-seeking behavior induced by an i.p. or i.v. priming injection in mice has not been clarified, it has been postulated that in contrast to rats, mice are sensitive to different discriminative stimulus effects or interoceptive cue(s) produced by an i.p. or i.v. priming injection (e.g. a more rapid onset of action with i.v. priming infusions vs. a slower one with i.p. priming injections), and the interoceptive cue provided by an i.p. priming injection may not be sufficiently associated with the previously experienced drug reinforcement via an i.v. infusion [6,48,85]. Although the subject-regulated injection of cocaine or a combination of a non-contingent i.v. infusion and a small number of self-administered infusions of METH reliably reinstated drug-seeking behavior in the 129 and C57BL/6J mouse strains [48,97], it should be noted that the drug-primed reinstatement behavior might reflect acute extinction behavior immediately after the cessation of the self-regulated drug infusion. Another drawback of these procedures is the relatively short patency of the jugular catheter in mice. Thus, we argue that these experimental designs are not optimal for drug-primed reinstatement behavior in mice. Interestingly, in recent studies [99,100], we have successfully demonstrated that an i.p. priming injection of METH dose-dependently reinstates drug-seeking behavior in wild-type C57BL/6 mice or glial cell-derived neurotrophic factor (GDNF) heterozygous knockout animals, by modifying the experimental procedures [99,100].

2.2.4. Dose-dependent curve and dosing order

Similar to drug SA, the magnitude of drug-primed reinstatement has been shown to be dose-dependent, with low doses producing a less robust reinstatement than moderate or high doses [48,70,99,100]. **Using a between-subjects design** for dose-dependent drug-primed reinstatement behavior in mice, the subgroup of animals for each dose and dosing order should be balanced across individual animals based on their drug intake during drug SA training, since our correlation study has revealed that the number of active nose-poke responses during the test for effective METH-primed reinstatement behavior (at 1.0 mg/kg of METH, i.p.) is positively correlated with the total amount of METH taken by individual mice during drug SA training [99].

Importantly, using a within-subjects design, the dosing order for the priming injection of addictive drugs may be critical for the successful observation of dose-dependent drug-primed reinstatement behavior in mice. The order of different doses for the priming injection of addictive drugs could be randomized by a Latin Squares design [48], or according to an ascending sequence from saline to progressively higher doses [39,99,100]. If the former dosing order is used to determine the dose-dependent curve for drug-primed reinstatement behavior in mice, it is essential to introduce a few sessions of extinction training between two tests at different doses of addictive drugs. In this case, a prolonged testing period may lead to a failure of transient drug-primed reinstatement behavior in mice [99]. Another limit is that repeated extinction training may attenuate the subsequent tests for drug-primed reinstatement behavior in animals, especially in mice [82]. Without the introduction of extinction training between two different doses, on the other hand, effects of a prior higher effective dose of drug will mask effects of the subsequent test dose. This is because addictive drug-reinstated drug-seeking behavior theoretically remains until, even beyond, the next test with a lower ineffective dose of drugs once the priming injection of additive drugs effectively reinstates drug-seeking behavior (e.g. a few days and beyond). In contrast, it is advisable for researchers to use the ascending sequence (from saline to progressively higher doses) for testing dose-dependent drug-primed reinstatement behavior in mice, during which a possible delay in drug-primed reinstatement behavior could be detected by consecutively testing for reinstatement behavior (without extinction training between two doses of drug priming injections) according to a within-subjects design [99].

2.2.5. Non-contingent priming infusions of drug solution prior to each session of drug SA training

A common practice in drug self-administration studies is to start the training sessions with one or two non-contingent priming infusions of addictive drug solution [73,85]. As in the case of prior operant training with natural rewards, this manipulation surely facilitates the acquisition of stable drug self-administration behavior, but it also confounds the interpretation of experimental data from subsequent tests for drug-primed reinstatement behavior.

When non-contingent priming infusions of an addictive drug solution are given at the start of each training session, they may become discriminative cues that predict drug availability [12]. As these priming infusions are not given during the extinction phase, when the priming injection is reintroduced during subsequent tests for drug-primed reinstatement behavior, it may reinstate drug-seeking because of its discriminative stimulus properties (i.e., it informs the animals that the drug is now available or that subsequent lever presses will lead to contingent drug infusions). Thus, when priming infusions of an addictive drug solution are given prior to each training session, their effects on reinstatement behavior after extinction may be due to their discriminative stimulus effects, incentive motivational effects, or both. To avoid confounding influences in studies of drug-primed reinstatement behavior, therefore, it would be optimal to avoid non-contingent priming infusions at the onset of each training session [73]. When researchers are not targeting drug-primed reinstatement behavior, or relapse to abuse of reinforcers such as nicotine, alcohol, and morphine, however, non-contingent priming injections of the addictive drug solution at the start of each training session may be a good alternative to operant training with natural rewards and/or food/water restriction.

Taken together, consistent with previous studies in rats [21,52], the reinstatement procedure itself (e.g. extinction conditions or extinction-reinstatement schedules) plays an important role in the successful observation of the drug-primed reinstatement of extinguished drug-seeking behavior in mice.

2.3. Cue-induced reinstatement behavior

It has long been recognized that drug-conditioned environmental stimuli are important determinants of drug-seeking behavior [22,32]. Accordingly, external stimuli (typically light and/or tones that have been repeatedly paired with drug delivery) can provoke reinstatement of extinguished operant responding when presented response-contingently or non-contingently during reinstatement testing [3,30,95]. One possible explanation for this effect is that the conditioned stimuli are acting as secondary reinforcers. A secondary reinforcer refers to a previously neutral stimulus (e.g. tone or light) that has acquired reinforcing properties through its prior association with a primary reinforcer (e.g. food or drugs) [12]. Therefore, operant responding during the test session may have been maintained by the cue's ability to function as a secondary reinforcer.

Similar to previous reports in rats [18,60] and monkeys [33], it has been reported that drug-associated cues reliably reinstate extinguished drug-seeking behavior in various mouse strains (including mutant animals) for different addictive substances such as cocaine, METH, alcohol, and nicotine [29,39,48,59,69,88,97,99–101]. Regardless of procedural factors, to date, cue-induced reinstatement behavior appears to be very robust in mice when compared with drug-primed or stress-triggered reinstatement behavior [29,39,48,59,69,88,97,99–101]. This procedure is expected to open a window to investigate roles of candidate genes in the secondary reinforcing properties of environmental cues previously associated with the delivery of drug solutions.

2.4. Stress-stimulated reinstatement behavior

Human and animal studies have suggested that stress is one of the most important factors in drug relapse after a period of abstinence [27,57,66,80]. **Although stress-triggered reinstatement** behavior has been widely investigated in rats, the data on stress-stimulated reinstatement behavior in mice are very limited [4,39].

Food deprivation has been regarded as a stressor because it activates the hypothalamic–pituitary–adrenal stress axis in animals [39,66]. As a slight stressor, food deprivation has been reported to reliably reinstate cocaine-seeking in the 129X1/SvJ strain, but the magnitude of operant responding is dependent on the level of food deprivation (22 h vs. 1 h deprivation) [39]. It has been reported that as a widely used stressor in animal models, foot shock is able to reinstate nicotine-seeking behavior in mouse individuals with high stress sensitivity, although there is no significant difference in acquisition and maintenance of nicotine consumption between individuals with high stress sensitivity and those with low stress sensitivity [4]. This suggests that a genetic predisposition to high stress sensitivity may specifically contribute to vulnerability to drug relapse. In humans, psychosocial stress elicits and increases the urge to smoke [24], and abstaining smokers report that they have stress [65] and difficulty dealing with stress [76]. Therefore, it could be worth extending the present model of stress-triggered relapse to other mouse strains and identifying specific genetic risk factors that may contribute to stress-triggered drug relapse.

3. Characterization

In the last decade, an SA–extinction-reinstatement procedure has been widely used in rats to elucidate potential factors and mechanisms underlying drug relapse [26,72,73]. Recently, efforts have been made at a similar procedure in mice. Based on documented reports and our own experiences, we try to summarize some characteristics of extinction-reinstatement behavior in mice as below (Table 2).

3.1. Extinction

3.1.1. Resistance to extinction

It has been reported that rats typically take about 6–10 daily sessions to extinguish cocaine-, METH-, heroin-, and nicotine-seeking behavior with a "between-session" extinction schedule [2,54]. Under similar conditions in mice, however, it has been reported to take 10–20 daily sessions to extinguish cocaine-, METH-, nicotine- or alcohol-seeking behavior [4,29,39,69,97,99–101]. In our studies of METH-seeking behavior, a few C57BL/6 mice took one month or more to lose METH-associated operant responding following METH (0.1 mg/kg/infusion) SA training (unpublished data). This phenomenon is consistent with previous observations of alcohol-seeking behavior in mice [101]. Interestingly, the resistance to extinction in mice is similar to the case in human addicts [13,14,31,34,38,58], **although the possible mechanism(s) underlying** the resistance to extinction in mice remains unclear. Some researchers argue that the increased extinction resistance in mice, as compared with that in rats, may result from species-specific hyperactivity, procedural difference, even difference in behavioral criteria, but do not imply that the mouse model of relapse possesses **stronger construct validity than the rat model** [29].

3.1.2. Attenuation of reinstatement behavior by repeated extinction training

Similar to the case in rats, repeated extinction training reduces the magnitude of subsequent reinstatement of drug-seeking behavior in mice [29,99,100]. We have reported a decrease in drug-seeking behavior induced by METH-associated cues with prolonged withdrawal and repeated sessions of extinction training in the wild-type C57BL/6 strain, GDNF heterozygous knockout animals, and GDNF wild-type littermates [99,100]. These observations seem inconsistent with previous reports that the magnitude of extinction responding and subsequent cue-induced reinstatement behavior often shows evidence for incubation of drug-craving behavior following a delay of several weeks in rats [55]. This discrepancy

Table 2
Characteristics of extinction-reinstatement behavior in rats and mice

Operant behavioral items	Rats	Mice
Duration of extinction training	Shorter	Longer [29,78,97,101]
Time-dependent incubation of drug craving	Yes	Not detected yet
Drug-primed reinstatement	Yes	Weak and transient [29,39,48,97,99]
Cue-induced reinstatement	Strong and long-lasting	Strong and long-lasting [29,39,97,99]
Stress-triggered reinstatement	Yes	Yes but limited [4,39]

may be because the extinction responding and subsequent cue-induced reinstatement behavior in our study were examined after repeated cycles of extinction training (a within-subjects design), since repeated training decreases the propensity for drug relapsing [82].

3.2. Drug-primed reinstatement

3.2.1. Weak effects of drug priming on reinstatement behavior

There are a few reports related to drug-primed reinstatement of drug-seeking behavior in mice. It has been reported that the i.p. priming injection of cocaine fails to reinstate drug-seeking behavior in the C57BL/6 and 129X1/SvJ strains [29,39]. The i.v. priming infusion of cocaine could reinstate drug-seeking behavior, but only at the highest dose examined (6.0 mg/kg) [39]. Recently, using a within-subjects design, it has been demonstrated that response-contingent access to cocaine dose-dependently reinstates drug-seeking behavior [48]. Under similar conditions, we have previously found that the i.p. priming of METH fails to reinstate drug-seeking behavior in the C57BL/6 strain, although a combination of non-contingent intravenous (i.v.) priming and self-injected METH increases reinstatement behavior in the absence of METH-paired stimuli (cue- and hole-lamps) and without METH infusion prior to self-injection [97]. However, we have recently found that using a within-subjects design, the i.p. priming injection of METH dose-dependently reinstated drug-seeking behavior in wild-type C57BL/6 mice, GDNF heterozygous knockout animals, and GDNF wild-type littermates, by improving the experimental procedures [99,100]. It should be noted that METH-primed reinstatement of drug-seeking behavior in GDNF wild-type littermates is weaker than that in the C57BL/6J strain [99,100]. Although it remains unclear whether the effects of drug priming injections on reinstatement of extinguished drug-seeking behavior are much weaker in mice than that in rats, these findings in mice suggest at least three points. First, the effects of priming injections of addictive drugs on the reinstatement of drug-seeking behavior are weaker in mice than in rats. Second, the drug-primed reinstatement of drug-seeking behavior in mice seems to be sensitive to experimental procedures including the route of drug priming administration, a within-subjects design, or a between-subjects design [99]. Third, similar to distinct drug SA behavior in various mouse strains, the drug-primed reinstatement of drug-seeking behavior is expected to be different in various mouse strains including inbred and outbred strains, since genetic background plays an important role in the development of drug dependence and relapse [68].

3.2.2. Transient drug-primed reinstatement behavior

Using a within-subjects design, we have recently found in the C57BL/6 strain that METH-primed reinstatement of extinguished drug-seeking behavior disappears within 2 months after withdrawal from METH self-administration. In contrast, cue-induced reinstatement of extinguished drug-seeking behavior lasts for at least 5 months after withdrawal from METH self-administration in wild-type C57BL/6 mice and GDNF heterozygous knockout animals, whereas the cue-induced reinstatement of extinguished drug-seeking behavior also disappears within half a year in GDNF wild-type littermates [99,100]. If this is the case for all addictive drugs, researchers may have to take experimental procedures into consideration during testing for drug-primed reinstatement of extinguished drug-seeking behavior in mice.

3.3. Cue-induced reinstatement

3.3.1. Strong effects of drug-associated cues on reinstatement behavior

In contrast to the weak effects of drug priming injections on drug-seeking behavior in mice, to date, all reports have indicated that drug-associated cues are so strong that cue-induced reinstatement behavior is readily observed in mice under different conditions including a between-subjects design or within-subjects design, a "between-session" schedule or "between-within-session" schedule, different types of addictive substances (e.g. cocaine, METH, alcohol), and various mouse strains (e.g. C57BL/6, 129X1/SvJ, mutant mouse strains, and their wild-type littermates) [29,39,48,59,69,88,97,99–101]. These findings suggest that the cue-induced reinstatement of drug-seeking behavior in mice is a reliable and useful model of relapse with which to identify specific genes involved in vulnerability to drug dependence/addiction in humans.

3.3.2. Long-lasting cue-induced reinstatement behavior

In contrast with the transient nature of METH-primed reinstatement behavior in mice, we have recently found that METH-associated cue-induced reinstatement behavior in the C57BL/6J strain or GDNF heterozygous knockout animals persisted for at least 5 months (presumably equivalent to 8–10 years in humans) [99,100]. Different from previous reports that cue-induced reinstatement often shows evidence for incubation of drug-craving behavior following a delay of several weeks in rats [55], there was a decrease in drug-seeking behavior induced by METH-associated cues with prolonged withdrawal and repeated sessions of extinction training in our studies. One plausible explanation for this discrepancy is that the cue-induced reinstatement behavior in our study was examined after repeated cycles of extinction training (a within-subjects design), since repeated training decreases the propensity for a relapse of extinguished drug-seeking behavior [82]. Another plausible explanation is that the session time for METH self-administration training was 3 h, whereas a longer time (e.g. prolonged access to addictive substances) seems to be critical for the demonstration of incubation of drug craving in rodents [52,55].

3.4. Stress-stimulated reinstatement

To date, there have been only two reports showing the stress-triggered reinstatement of drug-seeking behavior in mice [4,39]. The 129X1/SvJ mouse strain has been reported to show time-dependent reinstatement of extinguished cocaine-seeking

behavior after food deprivation (as mild stress) [39]. It was recently reported that footshock-triggered reinstatement of extinguished nicotine-seeking behavior appeared to depend on individual sensitivity to stress in the F2 generation from an intercross of high (C57BL/6J) and low (C3H/J) emotional mouse strains [4]. These findings suggest the effect of stress to be weak and limited when it comes to reinstating drug-seeking behavior in mice, as compared with the ability of stress to trigger drug-seeking behavior in rats. Furthermore, specific genes or genetic background are expected to be critical for stress-induced reinstatement of extinguished drug-seeking behavior [4]. In the near future, it would be interesting to extend the present findings of food deprivation or footshock-triggered reinstatement behavior to other mouse strains or other types of physical or psychological stress stimulation.

3.5. Correlation between drug SA, extinction, and triggering factors of reinstatement behavior

Similar to the case in rats [21,52,81], the amount of drug intake during drug SA training in mice should have profound impacts on subsequent tests for reinstatement behavior. It has been reported in the 129X1/SvJstrain that a high correlation ($r=0.7$) was observed between cocaine intake during training and the effect of priming with a high dose of cocaine on reinstatement behavior [39]. Recently, our correlation study has revealed in C57BL/6Jmice that there is a positive correlation between the total amount of METH taken by individuals during SA training and the magnitude of METH-primed reinstatement behavior [99]. However, there was no correlation between the total amount of METH taken by mice during SA and the number of active nose-poke responses during the test for cue-induced reinstatement, or the first session of extinction training, although individual mice show much variation in the number of extinction sessions required to achieve the extinction criteria [29,48]. Also, there was no correlation in the number of active nose-poke responses between the testing for METH-primed reinstatement and the testing for cue-induced reinstatement, probably suggesting that METH-primed and METH-associated cue-induced reinstatement involve different neural substrates or transmission pathways. In addition, it has been reported that foot shock-stimulated reinstatement of nicotine-seeking behavior in mice mainly depends on individual reactivity to stress rather than nicotine SA behavior [4], although in rats, the amount of total drug intake during drug SA training appears to influence the magnitude of reinstatement behavior induced by footshock stimulation [81].

There seems no positive correlation between the rate of operant responding during drug SA training and the magnitude of subsequent reinstatement behavior. It has been reported that under an FR1 schedule, the training dose of cocaine (0.2, 0.4, or 1.0 mg/kg/infusion), which leads to a high, moderate, or low rate of operant responding, respectively) does not alter the magnitude of reinstatement behavior induced by cocaine priming in rats [16]. In addition, regardless of the training dose, food restriction enhances the effect of cocaine priming on reinstatement behavior. It has been found that after SA reinforced by heroin (0.025, 0.05, 0.1, or 0.2 mg/kg/infusion); FR1 schedule) or cocaine (0.25, 0.5, 1.0, or 2.0 mg/kg/infusion); FR1 schedule) on alternate days, the rate of operant responding in rats during training (which is high with low doses and low with high doses) does not predict the magnitude of operant responding to cocaine or heroin priming after extinction training [53]. **In contrast, a previous report has shown** that the rate of operant responding during drug SA training is negatively correlated with the magnitude of cue-induced reinstatement [49].

4. Concluding remarks

Although available reports are limited on the extinction-reinstatement behavior in mice, there is an impetus for researchers to pursue mouse models at least based on the following two points: identification of potential genetic factors involved in drug relapse, and much similarity between the mouse model of extinction-reinstatement behavior and the observed characterizations of drug abstinence and relapse in human addicts. Mice with targeted gene mutations, such as transgenic and knockout mice, provide a powerful tool for investigating candidate genes/proteins that may be involved in drugs of abuse in humans [17,50,78]. Thus, it is likely that future studies using mouse models of reinstatement behavior will yield important information on the role of specific genes in drug-relapsing behavior. In addition, the magnitude of the reinstatement behavior induced by drug priming, drug-associated cues, and stress stimulation seems to decrease with the duration of withdrawal from drug SA ([99,100], and our unpublished observation). This phenomenon seems to be similar to the case in human addicts, in which the risk rate for drug relapse appears to decrease with the duration of abstinence from addictive drug-taking behavior [13,14,31,34,38,58]. In contrast, the time course of vulnerability to reinstatement behavior in rats is opposite to that of relapse risk in human addicts [55].

In addition to the SA–extinction-reinstatement procedure, other extensions of the drug SA model can also be used to identify specific risk genes/proteins involved in the end-stage of drug abuse. One example is that animal paradigms under a second-order schedule of reinforcement could be used to evaluate drug-seeking behavior in rats based on cocaine self-administration [3,78]. Another example is the "incubation" of drug-craving behavior in rats with an increase in the duration of withdrawal from cocaine SA [55]. A third example is escalated drug consumption in rats originally with prolonged access to cocaine through SA, and then being extended to other addictive drugs such as heroin, and METH [1,46,52]. A recent example of extension is addiction-like or compulsive drug-taking behavior in rats. After prolonged cocaine SA, cocaine-seeking behavior in rats becomes compulsive [20,92]. In the near future, these extensions of the drug SA model in genetically modified animals may help to identify potential specific genes/proteins involved in the late stages of drug dependence/addiction.

In conclusion, the SA–extinction-reinstatement procedure and distinct characteristics of drug-primed, cue-induced, and stress-triggered reinstatement of extinguished drug-seeking behavior in mice may provide a new platform for identifying specific genes/proteins involved in drug relapse by using genetically modified animals. Using this mouse model of relapse, several specific genes or molecules have been identified as important in vulnerability to drug relapse [59,69,100]. Future studies will need to extend the present SA–extinction-reinstatement procedure to additional mouse strains, as well as genetically manipulated animals. Further characterization of extinction and reinstatement behavior induced by various addictive substances in different mouse strains will increase our understanding of the genetic and neurobiological mechanism(s) underlying drug relapse in humans.

Acknowledgements

This study was supported in part by a Grant-in-aid for Health Science Research on Regulatory Science of Pharmaceuticals and Medical Devices, and for Research on Risk of Chemical Substances from the Ministry of Health, Labour and Welfare, Japan; by the Uehara Memorial Foundation; and by a Grant for the "Academic Frontier" Project for Private Universities (2007–2011) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

[1] Ahmed SH, Koob GF. Transition from moderate to excessive drug intake: change in hedonic set point. Science 1998;282:298–300.
[2] Anggadiredja K, Nakamichi M, Hiranita T, Tanaka H, Shoyama R, Watanabe S, et al. Endocannabinoid system modulates relapse to methamphetamine seeking: possible mediation by the arachidonic acid cascade. Neuropsychopharmacology 2004;29:1470–8.
[3] Arroyo M, Markou A, Robbins TW, Everitt BJ. Acquisition, maintenance and reinstatement of intravenous cocaine self-administration under a second-order schedule of reinforcement in rats: effects of conditioned cues and continuous access to cocaine. Psychopharmacology 1998;140:331–44.
[4] Bilkei-Gorzo A, Racz I, Michel K, Darvas M, Maldonado R, Zimmer A. A common genetic predisposition to stress sensitivity and stress-induced nicotine craving. Biol Psychiatry 2008;63:164–71.
[5] Bonson KR, Grant SJ, Contoreggi CS, Links JM, Metcalfe J, Weyl HL, et al. Neural systems and cue-induced cocaine craving. Neuropsychopharmacology 2002;26:376–86.
[6] Boyer CS, Petersen DR. Pharmacokinetic analysis of the metabolism of cocaine to norcocaine and N-hydroxynorcocaine in mice. Drug Metab Dispos 1992;20:863–8.
[7] Carney JM, Landrum RW, Cheng MS, Seale TW. Establishment of chronic intravenous drug self-administration in the C57BL/6J mouse. Neuroreport 1991;2:477–80.
[8] Carroll ME. The role of food deprivation in the maintenance and reinstatement of cocaine-seeking behavior in rats. Drug Alcohol Depend 1985;16:95–109.
[9] Carroll ME. Interaction between food and addiction. In: Niesnik RJM, Hoefakker RE, Westera W, Jaspers RMA, Kornet LMW, Boobis S, editors. Neurobiobehavioral toxicology and addiction: food, drugs and environment. Boca Raton, FL: CRC Press; 1999. p. 286–311.
[10] Carroll ME, Comer SD. Animal models of relapse. Exp Clin Psychopharmacol 1996;4:11–8.
[11] Carroll ME, Meisch ME. Increased drug-reinforced behavior due to food deprivation. In: Thompson T, Dews PB, Barrett JE, editors. Advances in behavioral pharmacology. New York: Academic Press; 1984. p. 47–88.
[12] Catania CA. Learning. 3rd ed. Englewood Cliffs, New Jersey: Prentice-Hall; 1992.
[13] Childress AR, McLellan AT, Ehrman RN, O'Brien CP. Extinction of conditioned responses in abstinent cocaine or opioid users. NIDA Res Monogr 1987;76:189–95.
[14] Childress AR, McLellan AT, O'Brien CP. Behavioral therapies for substance abuse. Int J Addict 1985;20:947–69.
[15] Cohen JB, Dickow A, Horner K, Zweben JE, Balabis J, Vandersloot D, et al. Methamphetamine Treatment Project: abuse and violence history of men and women in treatment for methamphetamine dependence. Am J Addict 2003;12:377–85.
[16] Comer SD, Lac ST, Wyvell CL, Curtis LK, Carroll ME. Food deprivation affects extinction and reinstatement of responding in rats. Psychopharmacology 1995;121:150–7.
[17] Crabbe JC, Phillips TJ, Harris RA, Arend MA, Koob GF. Alcohol-related genes: contributions from studies with genetically engineered mice. Addict Biol 2006;11:195–269.
[18] Davis WM, Smith SG. Role of conditioned reinforcers in the initiation, maintenance and extinction of drug-seeking behavior. Pavlovian J Biol Sci 1976;11:222–36.
[19] de Wit H, Stewart J. Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology (Berl) 1981;75:134–43.
[20] Deroche-Gamonet V, Belin D, Piazza PV. Evidence for addiction-like behavior in the rat. Science 2004;305:1014–7.
[21] Deroche-Gamonet V, Martinez A, Le Moal M, Piazza PV. Relationships between individual sensitivity to CS- and cocaine-induced reinstatement in the rat. Psychopharmacology (Berl) 2003;168:201–7.
[22] Di Ciano P, Blaha CD, Phillips AG. Changes in dopamine efflux associated with extinction, CS-induced and d-amphetamine-induced reinstatement of drug-seeking behavior by rats. Behav Brain Res 2001;120:147–58.
[23] Di Ciano P, Everitt BJ. Conditioned reinforcing properties of stimuli paired with self-administered cocaine, heroin or sucrose: implications for the persistence of addictive behaviour. Neuropharmacology 2004;47:202–13.
[24] Doherty K, Kinnunen T, Militello FS, Garvey AJ. Urges to smoke during the first month of abstinence: relationship to relapse and predictors. Psychopharmacology (Berl) 1995;119:171–8.
[25] Ehrman RN, Robbins SJ, Childress AR, O'Brien CP. Conditioned responses to cocaine-related stimuli in cocaine abuse patients. Psychopharmacology 1992;107:523–9.
[26] Epstein DH, Preston KL, Stewart J, Shaham Y. Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. Psychopharmacology 2006;189:1–16.
[27] Erb S, Shaham Y, Stewart J. Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. Psychopharmacology (Berl) 1996;128:408–12.
[28] Fuchs RA, Branham RK, See RE. Different neural substrates mediate cocaine seeking after abstinence versus extinction training: a critical role for the dorsolateral caudate-putamen. J Neurosci 2006;26:3584–8.
[29] Fuchs RA, See RE, Middaugh LD. Conditioned stimulus-induced reinstatement of extinguished cocaine seeking in C57BL/6 mice: a mouse model of drug relapse. Brain Res 2003;973:99–106.
[30] Gerber GJ, Stretch R. Drug-induced reinstatement of extinguished self-administration behavior in monkeys. Pharmacol Biochem Behav 1975;3:1055–61.
[31] Gilpin EA, Pierce JP, Farkas AJ. Duration of smoking abstinence and success in quitting. J Natl Cancer Inst 1997;89:526–72.
[32] Goldberg SR. Stimuli associated with drug injections as events that control behavior. Pharmacol Rev 1976;27:325–40.
[33] Goldberg SR, Kelleher RT, Goldberg DM. Fixed-ratio responding under second-order schedules of food presentation or cocaine injection. J Pharmacol Exp Ther 1981;218:271–81.
[34] Gossop M, Green L, Phillips G, Bradley B. Factors predicting outcome among opiate addicts after treatment. Br J Clin Psychol 1990;29:209–16.
[35] Gottfried JA, O'Doherty J, Dolan RJ. Encoding predictive reward value in human amygdala and orbitofrontal cortex. Science 2003;301:1104–7.
[36] Grant S, London ED, Newlin DB, Villemagne VL, Liu X, Contoreggi C, et al. Activation of memory circuits during cue-elicited cocaine craving. Proc Natl Acad Sci USA 1996;93:12040–5.
[37] Hester R, Garavan H. Executive dysfunction in cocaine addiction: evidence for discordant frontal, cingulate, and cerebellar activity. J Neurosci 2004;24:11017–22.
[38] Higgins ST, Badger GJ, Budney AJ. Initial abstinence and success in achieving longer term cocaine abstinence. Exp Clin Psychopharmacol 2000;8:377–86.
[39] Highfield DA, Mead AN, Grimm JW, Rocha BA, Shaham Y. Reinstatement of cocaine seeking in 129X1/SvJ mice: effects of cocaine priming, cocaine cues and food deprivation. Psychopharmacology 2002;161:417–24.
[40] Hunt WA, Barnett LW, Branch LG. Relapse rates in addiction programs. J Clin Psychol 1971;27:455–6.
[41] Kalivas PW, Volkow ND. The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 2005;162:1403–13.
[42] Kantak KM, Black Y, Valencia E, Green-Jordan K, Eichenbaum HB. Dissociable effects of lidocaine inactivation of the rostral and caudal basolateral amygdala on the maintenance and reinstatement of cocaine-seeking behavior in rats. J Neurosci 2002;22:1126–36.
[43] Katz JL, Higgins ST. The validity of the reinstatement model of craving and relapse to drug use. Psychopharmacology (Berl) 2003;168:21–30.
[44] Kendler KS, Jacobson KC, Prescott CA, Neale MC. Specificity of genetic and environmental risk factors for use and abuse/dependence of cannabis, cocaine, hallucinogens, sedatives, stimulants, and opiates in male twins. Am J Psychiatry 2003;160:687–95.
[45] Kilts CD, Gross RE, Ely TD, Drexler KP. The neural correlates of cue-induced craving in cocaine-dependent women. Am J Psychiatry 2004;161:233–41.
[46] Kitamura O, Wee S, Specio SE, Koob GF, Pulvirenti L. Escalation of methamphetamine self-administration in rats: a dose-effect function. Psychopharmacology (Berl) 2006;186:48–53.
[47] Kreek MJ, Nielsen DA, Butelman ER, LaForge KS. Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. Nat Neurosci 2005;8:1450–7.
[48] Kruzich PJ. Does response-contingent access to cocaine reinstate previously extinguished cocaine-seeking behavior in C57BL/6J mice? Brain Res 2007;1149:165–71.
[49] Kruzich PJ, Grimm JW, Rustay NR, Parks CD, See RE. Predicting relapse to cocaine-seeking behavior: a multiple regression approach. Behav Pharmacol 1999;10:513–21.
[50] Laakso A, Mohn AR, Gainetdinov RR, Caron MG. Experimental genetic approaches to addiction. Neuron 2002;36:213–28.
[51] Le DA, Li Z, Funk D, Shram M, Li TK, Shaham Y. Increased vulnerability to nicotine self-administration and relapse in alcohol-naive offspring of rats selectively bred for high alcohol intake. J Neurosci 2006;26:1872–9.
[52] Lenoir M, Ahmed SH. Heroin-induced reinstatement is specific to compulsive heroin use and dissociable from heroin reward and sensitization. Neuropsychopharmacology 2007;32:616–24.
[53] Leri F, Stewart J. Drug-induced reinstatement to heroin and cocaine seeking: a rodent model of relapse in polydrug use. Exp Clin Psychopharmacol 2001;9:297–306.
[54] Liu X, Caggiula AR, Palmatier MI, Donny EC, Sved AF. Cue-induced reinstatement of nicotine-seeking behavior in rats: effect of bupropion, persistence over repeated tests, and its dependence on training dose. Psychopharmacology (Berl) 2008;196:365–75.
[55] Lu L, Grimm JW, Hope BT, Shaham Y. Incubation of cocaine craving after withdrawal: a review of preclinical data. Neuropharmacology 2004;47:214–26.
[56] Mantsch JR, Baker DA, Francis DM, Katz ES, Hoks MA, Serge JP. Stressor and corticotropin releasing factor-induced reinstatement and active stress-related behavioral responses are augmented following long-access cocaine self-administration by rats. Psychopharmacology (Berl) 2008;195:591–603.
[57] Matheny KB, Weatherman KE. Predictors of smoking cessation and maintenance. J Clin Psychol 1998;54:223–35.
[58] McKay JR, Merikle E, Mulvaney FD, Weiss RV, Koppenhaver JM. Factors accounting for cocaine use two years following initiation of continuing care. Addiction 2001;96:213–25.
[59] Mead AN, Zamanillo D, Becker N, Stephens DN. AMPA-receptor GluR1 subunits are involved in the control over behavior by cocaine-paired cues. Neuropsychopharmacology 2007;32:343–53.

[60] Meil WM, See RE. Conditioned cued recovery of responding following prolonged withdrawal from self-administered cocaine in rats: an animal model of relapse. Behav Pharmacol 1996;7:754–63.
[61] Morgan AD, Carroll ME, Loth AK, Stoffel M, Wickman K. Decreased cocaine self-administration in Kir3 potassium channel subunit knockout mice. Neuropsychopharmacology 2003;28:932–8.
[62] Nestler EJ. Genes and addiction. Nat Genet 2000;26:277–81.
[63] O'Brien CP. A range of research-based pharmacotherapies for addiction. Science 1997;278:66–70.
[64] Panlilio LV, Yasar S, Nemeth-Coslett R, Katz JL, Henningfield JE, Solinas M, et al. Human cocaine-seeking behavior and its control by drug-associated stimuli in the laboratory. Neuropsychopharmacology 2005;30:433–43.
[65] Parrott AC, Kaye FJ. Daily uplifts, hassles, stresses and cognitive failures: in cigarette smokers, abstaining smokers, and non-smokers. Behav Pharmacol 1999;10:639–46.
[66] Piazza PV, Le Moal M. Pathophysiological basis of vulnerability to drug abuse: interaction between stress, glucocorticoids, and dopaminergic neurons. Annu Rev Pharmacol Toxicol 1996;36:359–78.
[67] Picciotto MR, Wickman K. Using knockout and transgenic mice to study neurophysiology and behavior. Physiol Rev 1998;78:1131–63.
[68] Ruiz-Durantez E, Hall SK, Steffen C, Self DW. Enhanced acquisition of cocaine self-administration by increasing percentages of C57BL/6J genes in mice with a nonpreferring outbred background. Psychopharmacology (Berl) 2006;186:553–60.
[69] Sanchis-Segura C, Borchardt T, Vengeliene V, Zghoul T, Bachteler D, Gass P, et al. Involvement of the AMPA receptor GluR-C subunit in alcohol-seeking behavior and relapse. J Neurosci 2006;26:1231–8.
[70] Schenk S, Partridge B. Cocaine-seeking produced by experimenter-administered drug injections: dose–effect relationships in rats. Psychopharmacology 1999;147:285–90.
[71] See RE. Neural substrates of cocaine-cue associations that trigger relapse. Eur J Pharmacol 2005;526:140–6.
[72] Shaham Y, Shalev U, Lu L, de Wit H, Stewart J. The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology (Berl) 2003;168:3–20.
[73] Shalev U, Grimm JW, Shaham Y. Neurobiology of relapse to heroin and cocaine seeking: a review. Pharmacol Rev 2002;54:1–42.
[74] Shelton KL, Beardsley PM. Interaction of extinguished cocaine-conditioned stimuli and footshock on reinstatement in rats. Int J Comp Psychol 2005;18:154–66.
[75] Shelton KL, Hendrick E, Beardsley PM. Interaction of noncontingent cocaine and contingent drug-paired stimuli on cocaine reinstatement. Eur J Pharmacol 2004;497:35–40.
[76] Siqueira LM, Rolnitzky LM, Rickert VI. Smoking cessation in adolescents: the role of nicotine dependence, stress, and coping methods. Arch Pediatr Adolesc Med 2001;155:489–95.
[77] Soria G, Mendizabal V, Tourino C, Robledo P, Ledent C, Parmentier M, et al. Lack of CB1 cannabinoid receptor impairs cocaine self-administration. Neuropsychopharmacology 2005;30:1670–80.
[78] Spanagel R, Sanchis-Segura C. The use of transgenic mice to study addictive behaviour. Clin Neurosci Res 2003;3:325–31.
[79] Spealman RD, Barrett-Larimore RL, Rowlett JK, Platt DM, Khroyan TV. Pharmacological and environmental determinants of relapse to cocaine-seeking behavior. Pharmacol Biochem Behav 1999;64:327–36.
[80] Stewart J. Pathways to relapse: the neurobiology of drug- and stress-induced relapse to drug-taking. J Psychiatry Neurosci 2000;25:125–36.
[81] Sutton MA, Karanian DA, Self DW. Factors that determine a propensity for cocaine-seeking behavior during abstinence in rats. Neuropsychopharmacology 2000;22:626–41.
[82] Sutton MA, Schmidt EF, Choi KH, Schad CA, Whisler K, Simmons D, et al. Extinction-induced upregulation in AMPA receptors reduces cocaine-seeking behaviour. Nature 2003;421:70–5.
[83] Szumlinski KK, Dehoff MH, Kang SH, Frys KA, Lominac KD, Klugmann M, et al. Homer proteins regulate sensitivity to cocaine. Neuron 2004;43:401–13.
[84] Thomsen M, Caine SB. Cocaine self-administration under fixed and progressive ratio schedules of reinforcement: comparison of C57BL/6J, 129X1/SvJ and 129S6/SvEvTac inbred mice. Psychopharmacology (Berl) 2006;184:145–54.
[85] Thomsen M, Caine SB. Intravenous drug self-administration in mice: practical considerations. Behav Genet 2007;37:101–18.
[86] Tran-Nguyen TL, Fuchs RA, Coffey GP, O'Dell LE, Baker DA, Neisewander JL. Time-dependent changes in cocaine-seeking behavior and dopamine overflow in the amygdala during cocaine withdrawal. Neuropsychopharmacology 1998;19:48–59.
[87] Trigo JM, Panayi F, Soria G, Maldonado R, Robledo P. A reliable model of intravenous MDMA self-administration in naive mice. Psychopharmacology (Berl) 2006;184:212–20.
[88] Tsiang MT, Janak PH. Alcohol seeking in C57BL/6 mice induced by conditioned cues and contexts in the extinction-reinstatement model. Alcohol 2006;38:81–8.
[89] Tsuang MT, Lyons MJ, Doyle T, Eisen SA, Goldberg J, True W, et al. Co-occurrence of abuse of different drugs in men: the role of drug-specific and shared vulnerabilities. Arch Gen Psychiatry 1998;55:967–72.
[90] Uhl GR. Needed: mouse/human cross validation of reinstatement/relapse models (and drug reward models) to model human substance abuse vulnerability allelic variants. Psychopharmacology (Berl) 2003;168:42–3.
[91] Uhl GR, Grow RW. The burden of complex genetics in brain disorders. Arch Gen Psychiatry 2004;61:223–9.
[92] Vanderschuren LJ, Everitt BJ. Drug seeking becomes compulsive after prolonged cocaine self-administration. Science 2004;305:1017–9.
[93] Volkow ND, Li TK. Drug addiction: the neurobiology of behaviour gone awry. Nat Rev Neurosci 2004;5:963–70.
[94] Weiss F, Maldonado-Vlaar CS, Parsons LH, Kerr TM, Smit DL, Ben-Shahar O, Control of cocaine-seeking behavior by drug-associated stimuli in rats: effects on recovery of extinguished operant-responding and extracellular dopamine levels in amygdala and nucleus accumbens. Proc Natl Acad Sci USA 2000;97:4321–6.
[95] Weiss F, Martin-Fardon R, Ciccocioppo R, Kerr TM, Smith DL, Ben-Shahar O. Enduring resistance to extinction of cocaine-seeking behavior induced by drug-related cues. Neuropsychopharmacology 2001;25:361–72.
[96] Wikler A. Dynamics of drug dependence, implication of a conditioning theory for research and treatment. Arch Gen Psychiatry 1973;28:611–6.
[97] Yan Y, Nitta A, Mizoguchi H, Yamada K, Nabeshima T. Relapse of methamphetamine seeking behavior demonstrated by a reinstatement procedure involving self-administration. Behav Brain Res 2006;168:137–43.
[98] Yan Y, Yamada K, Mizoguchi H, Noda K, Nagai T, Nitta A, et al. Reinforcing effects of morphine are reduced in tissue plasminogen activator-knockout mice. Neuroscience 2007;146:50–9.
[99] Yan Y, Yamada K, Nitta A, Nabeshima T. Transient drug-primed but persistent cue-induced reinstatement of extinguished methamphetamine-seeking behavior in mice. Behav Brain Res 2007;177:261–8.
[100] Yan Y, Yamada K, Niwa M, Nagai T, Nitta A, Nabeshima T. Enduring vulnerability to reinstatement of methamphetamine-seeking behavior in glial-cell-line-derived neurotrophic factor mutant mice. FASEB J 2007;21:1994–2004.
[101] Zghoul T, Abarca C, Sanchis-Segura C, Albrecht U, Schumann G, Spanagel R. Ethanol self-administration and reinstatement of ethanol-seeking behavior in Per1(Brdm1) mutant mice. Psychopharmacology (Berl) 2007;190:13–9.

Behavioural Brain Research 198 (2009) 172–178

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

The long-lasting effects of cross-fostering on the emotional behavior in ICR mice

Lingling Lu[a,b], Takayoshi Mamiya[a], Ping Lu[a,b], Minae Niwa[a,e], Akihiro Mouri[a,c], Li-Bo Zou[b], Taku Nagai[d], Masayuki Hiramatsu[a], Toshitaka Nabeshima[a,c,*]

[a] Department of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Meijo University, Japan
[b] Department of Pharmacology, School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, China
[c] Japanese Drug Organization of Appropriate Use and Research, Japan
[d] Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University Graduate School of Medicine, Japan
[e] Japan Society for the Promotion of Science, Japan

ARTICLE INFO

Article history:
Received 2 August 2008
Received in revised form 21 October 2008
Accepted 27 October 2008
Available online 6 November 2008

Keywords:
Cross-fostering
Early-life stress
Emotional behavior
Cognitive function
Serotonin
Mice

ABSTRACT

Early-life stress during the postnatal period could precipitate long-lasting alterations in the functional properties underlying emotional expression in humans, but how the psychological stress of cross-fostering affects emotional behavior during adulthood in mice remains primarily unknown. The purpose of the present study was to examine the long-term effects of cross-fostering on the emotional behavior and cognitive functions of ICR offspring in adulthood. Cross-fostering was performed from postnatal day 7 for 3 weeks. Mice were divided into three groups: (1) biological group: pups born from ICR dams fostered by their original mothers; (2) in-foster group: pups born from ICR dams but adopted by other ICR dams and (3) cross-foster group: ICR pups adopted by C57 dams. ICR mice were subjected to behavioral experiments at the age of 8 weeks. Emotional behaviors in the cross-fostered mice were significantly altered in the open-field, elevated plus maze and forced swimming tests, as well as social interaction tests. However, the cross-fostered mice showed normal memory function in the Y-maze and novel object recognition tests. The contents of serotonin metabolisms were decreased in the prefrontal cortex and hippocampus indicated the deficit of serotoninergic neuronal function by cross-fostering. These findings suggested that the early-life stress of cross-fostering induced long-lasting emotional abnormalities, which might be possibly related to alterations of serotonin metabolisms.

1. Introduction

Stressful events have been implicated in the onset or exacerbation of psychological disturbances in humans [2]. Exposure to adverse events early in life, such as childhood neglect and physical or sexual abuse is regarded as one of the most prominent environmental factors associated with the increased risk of emotional disorders [13]. Evidence is mounting to support the hypothesis that adverse early environments underlie vulnerability to a variety of psychological disorders, such as anxiety, depression and schizophrenia [6,7].

Early-life stress, during the prenatal or postnatal period, exerts lasting effects on neural development thus affecting behavior in rodents [1,33]. For instance, rats exposed to prenatal stress in utero exhibit increases in anxiety or depression-like behaviors [20,36] and impaired cognitive function with aging [33]. Prenatal stress also induces long-term changes in neurobiological systems, including hyperactivity of the hypothalamo-pituitary-adrenal (HPA) axis in response to later stress [20]. Changes of postnatal interactions with pups and dams could-profoundly affect the emotionality as well as cognition of offspring in rats too [3]. It has been reported that maternal deprivation for long periods during the first 3 weeks of life impairs emotional behavior and affects pyramidal dendritic outgrowth in the prefrontal cortex [29]. In contrast, some postnatal manipulations have opposite effects on the development of offspring. Repeated maternal separation for a period of 15 min each day for 3 weeks, known as postnatal handling, has anxiolytic properties, which reduces anxiety-like behavior in adult rats [36], improves the performance of aged offspring in cognitive tasks [17], and attenuates stress-induced secretion of corticosterone.

Cross-fostering as a kind of postnatal psychological stress, could modify the mother–infants relationship early in life and mimic the psychology of childhood adoption which has frequently happened recently. Clinical researchers have reported that adopted children with a history of prenatal substance exposure [4,28] or placed relatively late in their adoptive home are at heightened

* Corresponding author at: Department of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya 468-8503, Japan. Tel.: +81 52 839 2735; fax: +81 52 839 2738.
E-mail address: tnabeshi@ccmfs.meijo-u.ac.jp (T. Nabeshima).

0166-4328/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2008.10.031

risk of social, intellectual, and emotional problems [32]. Animal studies have also shown different responses of pups to adoption caused by differences in maternal care: BALB/cByJ dams displayed less nursing and licking/grooming of pups and spent less time in the nest than C57BL/6ByJ dams [10,11,30]. Other researchers have examined the influence of the interaction between genetic susceptibility and environmental factors on emotional behavior and reported that cross-fostering affects the level of anxiety in rats [12,39]. These findings indicate that cross-fostering may affect the behavior of offspring. However, most researchers have focused on the differences in maternal care between several species. It remains to be determined whether cross-fostering has long-lasting effects on emotionality as well as cognition in offspring during adulthood in ICR mice.

In the present study, to systematically investigate long-term effects of cross-fostering on emotional and cognitive functions in offspring, we examined emotional behavior, response to stress, social interaction, and cognitive function in adult ICR mice which had experienced cross-fostering for 3 weeks.

2. Materials and methods

2.1. Animals

Pregnant ICR and C57BL/6Jjms Slc dams (E12) obtained from SLC Japan (Shizuoka, Japan) were maintained on a 12/12-h light/dark cycle (lights on from 08:00 to 20:00) with free access to food (CE2; Clea Japan Inc., Tokyo, Japan) and water. Dams were housed individually till parturition. ICR pups were weaned at 28 days of age and housed by sex in each group. Male pups were used for behavioral analyses at the age of 8–9 weeks. All of the behavioral experiments were carried out in a sound-attenuated and air-conditioned experimental room (23 ± 1 °C, 50 ± 5% humidity). The mice were habituated for at least 30 min before the tests and all behavioral tests were recorded by DVD camera to reconsider these results. The experiments were performed in accordance with the Guidelines for Animal Experiments of Meijo University and the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society (2007).

2.2. Cross-fostering procedure

Cross-fostering was performed at the postnatal day 7 (PD7) and completed within 5 min. Litters which consisted of 10 pups per dam with an equal number of males and females were used when possible. In this way, mice were divided into three different groups: (1) biological group: pups born from ICR dams and fostered by their original mothers; (2) in-foster group: pups born from ICR dams but adopted by other ICR dams and (3) cross-foster group: ICR pups adopted by C57 dams. All of the pups in each litter were taken out from their original cages and shortly separated from their original dams. Then, for the biological group, pups in the same litters were returned to their own dams one by one; for the in-foster group, the pups were put into their adopted dams within the same strain; while for the cross-fostered pups, they were put into other cages of C57 dams. All of the litters were composed of pups with same development history. Each group for each time contained more than three litters and were repeated more than 3 times. All of the pups were weighed once a week from birth to 8 weeks old. Male offspring were randomly used to check the behavioral changes and biochemical analyses.

2.3. Open-field test

As previously described [23], the apparatus for the open-field test consisted of a square area with black walls (*L* 40 cm × *W* 40 cm × *H* 40 cm) and was set in the experimental room. The floor of the field was divided into 64 identical squares (16 center squares, 16 corner squares and 32 other squares) so that the ambulation of mice could be measured. A light (100 W bulbs) was positioned 100 cm above the center. Each mouse was placed in the same corner square of the apparatus and allowed to explore freely for 5 min. During this period, the latency to cross the center squares, the time spent in the center and the corner squares, the numbers of ambulation, rearing and grooming events, and the frequency of defecation as well as urination were counted [30]. To count ambulation or rearing, entry into a square was defined as all four legs being inside the square. At the end of the test, the mouse was returned to its home cage and the apparatus was thoroughly cleaned with 70% ethanol.

2.4. Elevated plus maze test

The elevated plus maze was made of wood and consisted of two open arms (*L* 25 cm × *W* 8 cm) and two closed arms (*L* 25 cm × *W* 8 cm × *H* 20 cm) emanating from a common central platform (8 cm × 8 cm) to form a plus shape. The entire apparatus was elevated to a height of 50 cm above the floor. Testing commenced by placing a mouse on the central platform of the maze facing an open arm, and the standard 5-min test duration was employed. An entry was defined by all four legs entered into the arm. The open arm entries (%) and the time spent in open arms (%), and the total arm entries were calculated. After each test, the apparatus was thoroughly cleaned with 70% ethanol as previously described [40].

2.5. Forced swimming test

Mice were placed individually in a transparent polycarbonate cylinder (*ϕ* 8 cm × *H* 20 cm) containing water at 22 °C to a depth of 11.5 cm, and forced to swim for a 5-min period. The duration of immobility behavior was measured automatically by a SCANET MV-20 (Melquest Co. Ltd., Toyama, Japan), as described previously [27].

2.6. Social interaction test

The apparatus for the social interaction test was made of a gray polycarbonate (*L* 30 cm × *W* 25 cm × *H* 25 cm) [31]. Lighting in the experimental room consisted only of a dark light (25 W bulbs) and was diffused to minimize shadows in the arena. Before the test, each mouse was habituated alone in the apparatus for 10 min on two consecutive days. On the test day, the mice were randomly assigned according to gender to an unfamiliar partner in each group. The pairs of unfamiliar mice were placed in the apparatus for 10 min and the total amount of time spent in active social interaction, such as sniffing, grooming, following and mounting as well as crawling over or under the partner, was recorded. Passive contact (sitting or lying with bodies in contact) was not included in social interaction. At the end of the test, all the boluses were removed and the apparatus was cleaned with 70% ethanol.

2.7. Y-maze test

The Y-maze apparatus was made of wood and consisted of three arms (*L* 40 cm × *W* 12 cm × *H* 3 cm at bottom, *L* 40 cm × *W* 12 cm × *H* 10 cm at top) which converged at equal angles. Mice were placed at the center of the apparatus and allowed to move freely through the maze during the 8-min session. The series of arm entries was recorded visually. Alternation was defined as successive entry into the three arms on overlapping triplet sets. Alternative behavior (%) was calculated as the ratio of actual alternations to possible alternations (defined as the number of arm entries minus two) multiplied by 100 [25].

2.8. Novel object recognition test

The novel object recognition test was performed following previous reports [21]. The test procedure consisted of three sessions: habituation, training, and retention. Each mouse was individually habituated to the box (*L* 30 cm × *W* 30 cm × *H* 30 cm), with 10 min of exploration in the absence of objects for 3 days (habituation session). During the training session, two objects were placed in the back corner of the box. A mouse was then placed midway at the front of the box and the total time spent exploring the two objects was recorded for 10 min. During the retention session, animals were placed back into the same box 24 h after the training session, in which one of the familiar objects used during training was replaced with a novel object. The animals were then allowed to explore freely for 5 min and the time spent exploring each object was recorded. Throughout the experiments, the objects were used in a counterbalanced manner in terms of their physical complexity and emotional neutrality. A preference index, the ratio of time spent exploring either of the two objects (training session) or the novel object (retention session) over the total amount of time spent exploring both the objects, was used to assess cognitive function.

2.9. Determination of monoamine and its metabolite levels in the brain

The prefrontal cortex and hippocampus were dissected out from the brains on an ice-cold plate immediately after the mice were decapitated. Each part of brain sample was quickly frozen and stored in a deep freezer at −80 °C until assayed. The contents of norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were determined using high-performance liquid chromatography with electrochemical detection [41].

2.10. Statistical analysis

All data were expressed as the means ± S.E.M. The analysis of body weight during the period of cross-fostering was conducted with a two-way analysis of variance (ANOVA), followed by Bonferroni's test as a *post hoc* comparison. Other statistical differences were tested using a one-way ANOVA followed by Bonferroni's test. A probability level of $P < 0.05$ was regarded as statistically significant.

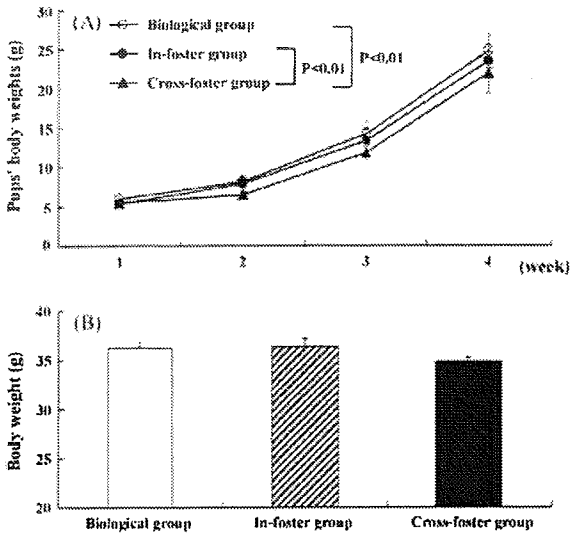

Fig. 1. Effect of cross-fostering on body weight. The body weight of pups during the cross-fostering period from 1 to 4 weeks (A) and at the age of 8 weeks (B). Data are expressed as the mean ± S.E.M. for 10–18 mice (Bonferroni's test).

3. Results

3.1. Effect of cross-fostering on body weight

As reported previously, body weight was considered as an assessment of cross-fostering, since it was sensitive to a change of rearing conditions [12]. To confirm the effect of cross-fostering, the weight of pups was measured during the period of cross-fostering and throughout their development. As shown in Fig. 1, the pups in the in-foster group gained weight as observed in the biological group. However, body weight was significantly lower in the cross-foster group than the biological or in-foster group during the period of cross-fostering ($F_{group(2,168)} = 22.40$, $P < 0.01$; $F_{week(3,168)} = 1394.66$, $P < 0.01$; $F_{group \times week(6,168)} = 2.13$, $P > 0.05$, Fig. 1A). No significant differences were observed among the three groups at the age of 8 weeks when all of the behavioral tests were started ($F_{(2,44)} = 1.80$, $P > 0.05$, Fig. 1B).

3.2. Effect of cross-fostering on behavior in the open-field test

An open-field test under mild stressful conditions is commonly used to detect emotional changes in mice [40]. In-fostered mice showed no changes of behavior in the open-field test compared with biological mice (Fig. 2). Meanwhile, the mice in the cross-foster group spent less time in the center squares ($F_{(2,43)} = 11.87$, $P < 0.01$, Fig. 2B) but longer in the corners ($F_{(2,43)} = 3.61$, $P < 0.05$, Fig. 2C) than either the biological or in-foster group when exposed to a novel environment under mild stressful conditions in the open-field. Furthermore, cross-fostered mice showed significant decreases in ambulation ($F_{(2,43)} = 5.26$, $P < 0.01$, Fig. 2D) and rearing ($F_{(2,43)} = 7.03$, $P < 0.01$, Fig. 2E) compared with the in-foster group. There were no significant differences in the latency to the center squares ($F_{(2,43)} = 0.37$, $P > 0.05$, Fig. 2A), the number of grooming events ($F_{(2,43)} = 0.55$, $P > 0.05$, Fig. 2F), and the frequency of defecation ($F_{(2,43)} = 0.12$, $P > 0.05$, Fig. 2G) as well as urination ($F_{(2,43)} = 0.72$, $P > 0.05$, Fig. 2H) in the cross-foster group (Fig. 2).

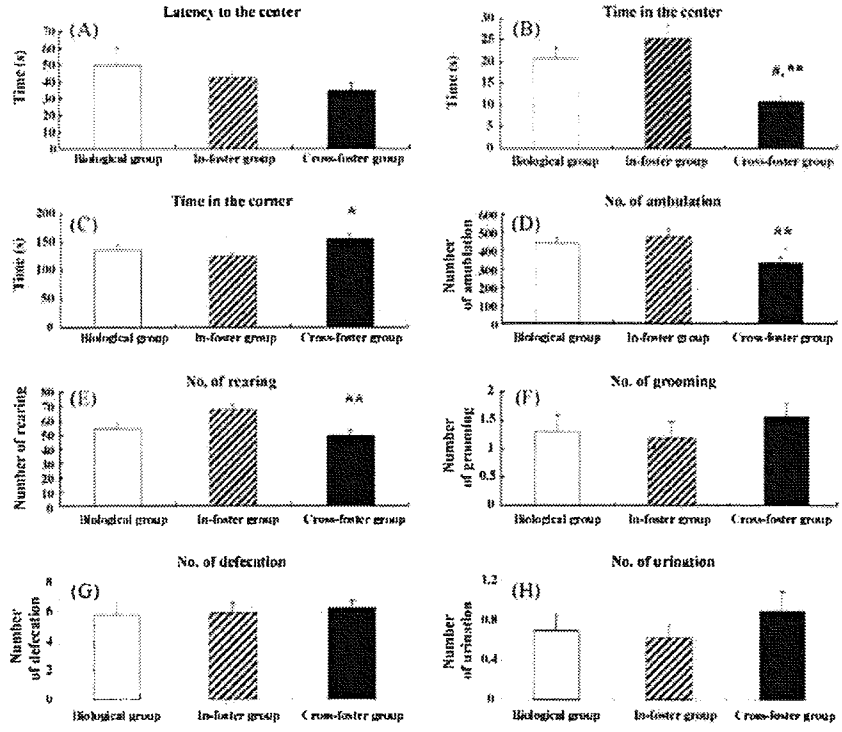

Fig. 2. Effect of cross-fostering on behavior in the open-field test. Time of latency to the center (A). Time spent in the center (B). Time spent in the corner (C). The number of times ambulation occurred (D). The number of times rearing occurred (E). The number of times grooming occurred (F). The number of times defecation occurred (G). The number of times urination occurred (H). Data are expressed as the mean ± S.E.M. for 10–18 mice. *$P < 0.05$, **$P < 0.01$ vs. in-foster group; #$P < 0.05$ vs. biological group (Bonferroni's test).

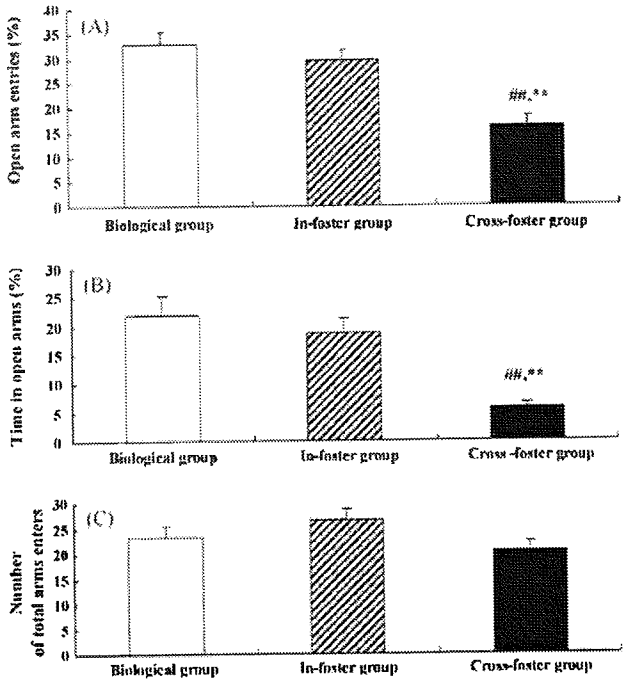

Fig. 3. Effect of cross-fostering on the behavior in the elevated plus maze test. Relative percentage of open arm entries (A). Relative percentage of time spent in open arms (B). Total number of arm entries (C). Data are expressed as the mean ± S.E.M. for 10–18 mice. $^{**}P<0.01$ vs. in-foster group mice; $^{\#\#}P<0.01$ vs. biological group (Bonferroni's test).

3.3. *Effect of cross-fostering on behavior in the elevated plus maze*

In the in-foster group, no significant changes in behavior in the elevated plus maze were observed compared with that in the biological group (Fig. 3). The percentage of open arm entries was significantly decreased in the cross-foster group compared with both the biological and the in-foster group ($F_{(2,44)}=15.90$, $P<0.01$, Fig. 3A). Notably, the percentage of time spent in the open arms was remarkably reduced in the cross-foster group compared with the other two control groups ($F_{(2,44)}=14.06$, $P<0.01$, Fig. 3B) whereas total arm entries was unaffected ($F_{(2,44)}=2.79$, $P>0.05$, Fig. 3C).

3.4. *Effect of cross-fostering on immobility time in the forced swimming test*

To further investigate the emotional response to stress, we examined the effect of cross-fostering on forced swimming-induced immobility. As shown in Fig. 4, the cross-fostered mice showed a significant increase of immobility time in the first 5 min

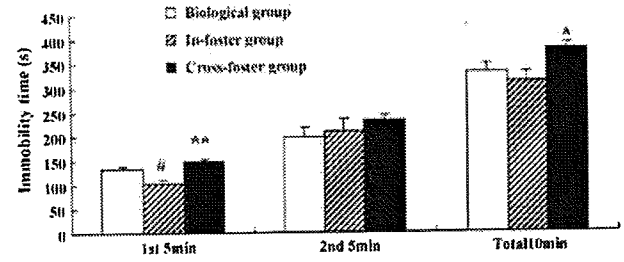

Fig. 4. Effect of cross-fostering on immobility time in the forced swimming test. Data are expressed as the mean ± S.E.M. for 10–17 mice. $^{*}P<0.05$, $^{**}P<0.01$ vs. in-foster group; $^{\#}P<0.05$ vs. biological group (Bonferroni's test).

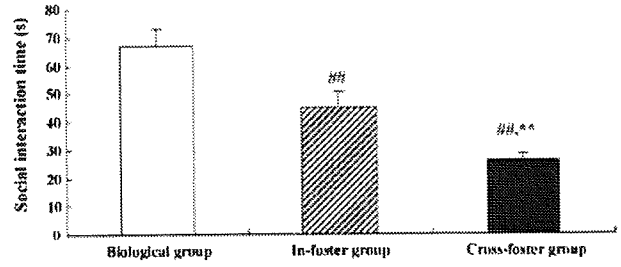

Fig. 5. Effect of cross-fostering on social interaction. Active social interaction behaviors, such as sniffing and grooming the partner, following, mounting, and crawling under or over the partner were recorded as the time of interaction during this period. Data are expressed as the mean ± S.E.M. for 10–17 mice. $^{**}P<0.01$ vs. in-foster group; $^{\#\#}P<0.01$ vs. biological group (Bonferroni's test).

and total 10 min but not the second 5 min (first 5 min: $F_{(2,42)}=9.06$, $P<0.01$; second 5 min: $F_{(2,42)}=0.74$, $P=0.48$, $P>0.05$; total 10 min: $F_{(2,43)}=4.88$, $P<0.05$, Fig. 4), compared with the in-fostered mice, which implied a state of increased depression which affected adaptation to stress. On the contrary, in-fostered mice showed a significant reduction of immobility time compared with biological control mice in the first 5 min, but not the second 5 min and total 10 min ($P<0.05$, Fig. 4).

3.5. *Effect of cross-fostering on social interaction*

The in-foster group showed a significant decrease in social interaction behavior compared with the biological group (Fig. 5). In addition, social interaction time was significantly shorter in the cross-foster group than the biological and in-foster groups ($F_{(2,42)}=17.84$, $P<0.01$, Fig. 5).

3.6. *Effect of cross-fostering on behavior in the Y-maze test*

There were no significant differences in spontaneous alternation behavior among the biological, in-fostered and cross-fostered mice in the Y-maze test (Fig. 6). The total number of arm entries was also unchanged among the three groups ($F_{(2,44)}=0.40$, $P>0.05$; $F_{(2,44)}=2.75$, $P>0.05$, respectively, Fig. 6).

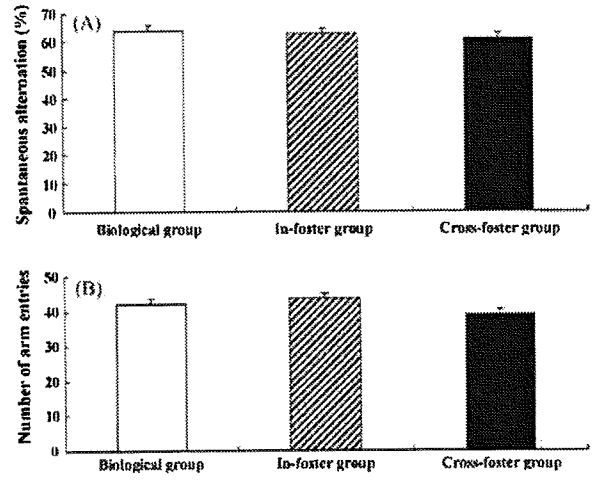

Fig. 6. Effect of cross-fostering on behavior in the Y-maze test. The percentage of spontaneous alternation behavior (A). The number of arm entries (B). Data are expressed as the mean ± S.E.M. for 10–18 mice. There were no significant differences among the groups (Bonferroni's test).

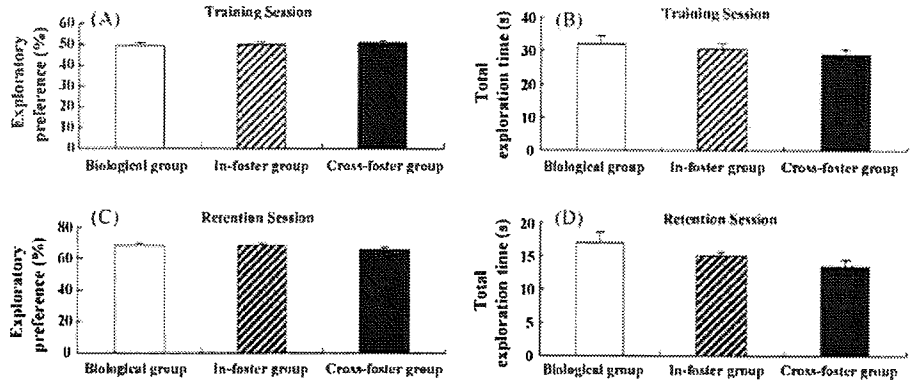

Fig. 7. Effect of cross-fostering on performance in the novel object recognition test. The percentage of exploratory preference in the training session (A). Total exploratory time in the training session (B). The percentage of exploratory preference in the retention session (C). Total exploratory time in the retention session (D). Data are expressed as the mean ± S.E.M. for 10–18 mice. There were no significant differences among the groups (Bonferroni's test).

3.7. Effect of cross-fostering on performance in the novel object recognition test

In the training session, the mice in the biological, in-foster and cross-foster groups spent equal amounts of time exploring either of the two objects ($F_{(2,44)} = 0.21$, $P > 0.05$, Fig. 7A), and thus there was no biased exploratory preference in either group of animals. In addition, total time spent in the exploration of objects in the training session did not differ among the three groups ($F_{(2,44)} = 0.55$, $P > 0.05$, Fig. 7B).

When retention performance was tested 24 h after the training session, three were no differences in the level of exploratory preference for the novel objects among the three groups ($F_{(2,44)} = 0.93$, $P > 0.05$, Fig. 7C). The total exploration time did not differ among the three groups in the retention session either ($F_{(2,44)} = 2.55$, $P > 0.05$, Fig. 7D).

3.8. Alteration of monoamine metabolism in the prefrontal cortex and hippocampus

To clarify the neurochemical basis of altered emotional behavior in cross-fostered mice, the amount of monoamines and their metabolites in the prefrontal cortex and hippocampus were determined. As shown in Fig. 8A, a significant decease of NE, 5-HT and its metabolites 5-HIAA as well as DA in the prefrontal cortex in cross-foster group was observed, compared with those in biological group (NE: $F_{(2,22)} = 3.93$, $P < 0.05$; 5-HT: $F_{(2,22)} = 3.80$, $P < 0.05$; 5-HIAA: $F_{(2,22)} = 6.07$, $P < 0.01$; DA: $F_{(2,22)} = 4.48$, $P < 0.05$, Fig. 8A). But, there were no differences for the contents of dopamine metabolites among groups, including DOPAC and HVA (DOPAC: $F_{(2,22)} = 1.16$, $P > 0.05$; HVA: $F_{(2,22)} = 0.94$, $P > 0.05$, Fig. 8A). In the hippocampus, compared with the biological group, the mice in cross-foster group also showed significant reduction of 5-HIAA ($F_{(2,25)} = 7.00$, $P < 0.01$, Fig. 8B), but no changes in NE, 5-HT, DA, DOPAC and HVA (NE: $F_{(2,25)} = 0.88$, $P > 0.05$; 5-HT: $F_{(2,25)} = 2.06$, $P > 0.05$; DA: $F_{(2,22)} = 2.92$, $P > 0.05$; DOPAC: $F_{(2,25)} = 0.63$, $P > 0.05$; HVA: $F_{(2,25)} = 1.87$, $P > 0.05$, Fig. 8B). For the in-foster group, the contents of NE in the prefrontal cortex and 5-HIAA in the hippocampus were also reduced compared with biological group (Fig. 8A and B). Whereas, the turnovers of monoaminergic neuronal systems were not affected by in- and cross-fosterings (data were not showed).

4. Discussion

Cross-fostering as a kind of early-life stress in rodents could mimic the psychology of children adopted as babies or suffering neglect as well as physical abuse [34]. Although most adopted individuals are well adjusted, population-based studies have reported an elevated risk for psychological maladjustment in adopted children compared with representative samples of nonadopted children [15]. A meta-analysis of findings from more than 25,000 adoptees, revealed significantly more behavioral and emotional problems among adoptees than nonadoptees [38]. Studies in animal models have found that cross-fostering within 24 h after birth affects the maternal behavior and pups' responses [11,30]. But, few articles have systemically examined its long-lasting effects on emotional or cognitive functions in ICR mice.

Clinical researches have reported that approximately 120,000 children in the USA are adopted annually, and adopted individuals constitute about 1.5 million children at young age [26]. However, the face of adoption is changing from decreasing domestic adoptions to a sharp increasing of international ones. Worldwide, approximately 40,000 children per year are moved between more than 100 countries through adoption [14]. Therefore, there is a persistent concern that adopted children may be at heightened risk for mental health or adjustment problems [16]. To clarify this concern, we designed the in- and cross-fostering groups to be equivalent to

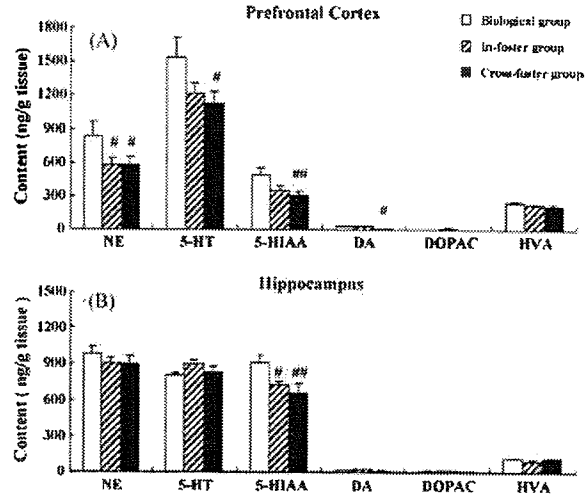

Fig. 8. Monoamines and their metabolite contents in the prefrontal cortex and hippocampus. The contents of monoamines and their metabolites in the prefrontal cortex (A). The monoamines and their metabolite contents in the hippocampus (B). Data are expressed as the mean ± S.E.M. for 8–10 mice. $^{**}P < 0.01$ vs. in-foster group; $^{*}P < 0.05$, $^{\#\#}P < 0.01$ vs. biological group (Bonferroni's test).

'the domestic' and 'international adoptions', respectively. Furthermore, we also think the degree of stress induced by adoption might be different for both pups and their adopted dams from in-fostering and biological groups. Therefore, it might produce different effects on long-term behavioral abnormality in each group. Some of our results showed these differences in behaviors and neurochemical parameters among them.

Previous studies have shown that adoption at different points in postnatal period affect the responses of pups and dams in rats: Early adoption on PD1 prevents the stress-induced secretion of corticosterone which is observed in offspring separated early, reduces locomotor activity in a novel environment, and improves spatial recognitive function [3,8]. In contract, later adoptions (PD5 and PD12) prolong the stress-induced secretion of corticosterone, increase locomotor response to novelty, and disrupt spatial recognition [3,8]. Furthermore, the second postnatal week has been reported as critical to establish the proper responses to later stress in adolescence [22]. Therefore, cross-fostering was carried out from PD7 to P28 in the present study to focus on the emotional behavior of offspring in adulthood.

Body weight during development has been considered as an indicator of maternal rearing [12]. In this study, we measured the body weight of pups throughout their development. No significant differences were observed at the age of 8 weeks when all of the behavioral tests were started. Therefore, it is unlikely that the behavioral changes in cross-fostered mice are due to malnutrition in adulthood. On the contrary, the body weight of ICR pups was reduced by cross-fostering during the period of adoption, indicating the maternal rearing by C57BL/6 dams is impaired by the adoption of a different strain of pups and the ICR pups is sensitive to adoption by C57BL/6 dams. Other possible explanations for the decrease in pups' body weight include the number of litters, the quality of adopted maternal care and the nourishment in milk, etc. [37], since we have used different strain of mice and alternated the relationship of dam and pups during their developing period. In this paper, we merely want to show the final co-effects of these factors in the first step of experiments. Therefore, we did not separate the exact role for each factor. Further researches are needed to clarify whether each factor differently influences on body weight in cross-fostering.

The changes in emotional behavior of mice were examined by the open-field, elevated plus maze and forced swimming as well as social interaction tests. In the open-field test, the mice are exposed to aversive stimuli (novelty, lit, and open area) to avoid and new places to explore. The internal conflict is measured by the duration of stay in peripheral areas and avoidance of the center area, called thigmotaxis, and the number of ambulation or rearing events is thought to reflect the exploratory tendencies in mice [35]. In the present study, the cross-fostered mice showed an increase in thigmotaxis and decrease in exploratory tendencies. The results were consistent with the previous report that C57BL/6ByJ mice raised by BALB/cByJ dams spent less time in the center of the open-field area [30]. Furthermore, in the elevated plus maze test, the cross-fostered mice showed a decrease in the both of the number of open arm entries and the time spent in open arms, parameters of potential anxiety in mice [18]. Taken together, these behavioral studies suggested the cross-fostered mice are in a state of increased anxiety. Immobility time in forced swimming test is used to evaluate the state of stress or depression since it can be increased by stressors and reversed by some antidepressants [9]. The cross-fostered mice showed a significant enhancement of immobility time during the first 5 min in the forced swimming test which suggests increased sensitivity to stress or inappropriate coping responses when facing severely stressful situations. Social withdrawal is regarded as a feature of emotional disorders [19]. We examined the social behavior of offspring in adulthood. The social interaction time in the cross-foster group was remarkably shorter than that in the in-foster group. Interestingly, the mice not only in cross-foster group but also in-foster group showed a significant decrease in social interaction behavior compared with those in the biological control group, suggesting the social interaction test may be more sensitive for detecting the behavioral changes of fostering. Taken together, all of the behavioral results indicate that the cross-fostered mice were in a state of high emotionality and so failed to adapt to other stressors.

Cognitive function was also evaluated by measuring the spontaneous alternation behavior of mice in the Y-maze test (an index of spatial memory) and exploratory preference in the novel object recognition test (an index of visual recognition memory) [25]. There were no significant differences in cognitive function among the different fostering groups suggesting that the cross-fostered mice were normal in cognitive function including short-term memory and visual recognition memory in ICR mice. However, the data supporting unaffected memory are inconclusive: later adoption from PD5 or PD12 has been reported to impair memory in the Y-maze test for male adult offspring in rats, whereas early adoption from PD1 has the opposite effect [3]. The differences may be partly due to either the period of cross-fostering or the strains of mice in each study. Therefore, we cannot make such conclusion that the stress of cross-fostering might not be strong enough to impair learning or memory in ICR mice. Other types of memory should be tested such as spatial memory, contextual memory, latent learning associated with selective attention and motor learning.

In the present study, we used the same offspring for behavioral tests, repeatedly, to measure various emotional and cognitive functions. It is unlikely that the carry-over effects of previous behavioral tests would affect the following behavioral tests, since (1) each behavioral test consists of different parameters which affect motivation and curiosity, etc., (2) all groups have the same influences from previous behavioral tests and (3) we compared them with the control one. The control group in the present study did not show any behavioral abnormality compared with that in our previous studies [23,25,40].

To investigate the neurochemical basis of these emotional abnormalities, we measured the levels of monoamine neurotransmitters and their metabolites in the prefrontal cortex and hippocampus, which related to emotional and cognitive function [40]. We found the contents of 5-HT and 5-HIAA were reduced in cross-fostered mice, indicating the deficit of serotoninergic neuronal function by cross-fostering. It is well known the serotonergic system plays a critical role in regulation of emotional stress during development, and the dysfunction of serotonergic system has been implicated in the etiology of emotional disorders [16,22]. Therefore, the aberrant serotonergic system or its receptors induced by cross-fostering may lead to these emotional abnormalities. Furthermore, since the dysfunctions of noradrenergic and dopaminergic system are also related to some stress-induced disorders, such as depression [5,24], the reduction of NE and DA in the prefrontal cortex might partly contribute to these emotional abnormalities of cross-fostered mice in the present study. However, further research is needed to determine the precise mechanisms of cross-fostering on impaired emotional behavior in ICR mice.

5. Conclusion

In conclusion, the present study demonstrated that cross-fostering of ICR pups with C57BL/6 dams from PD7 for 3 weeks affected the emotionality, but not memory, of offspring in adulthood which could mimic the psychology of adoption in humans. Furthermore, stress-related psychological diseases are known to

involve both genetic susceptibility and environmental factors, but the interactions of genes and the environment in the susceptibility to stress are still unclear [7]. The early-life stress induced by cross-fostering has important implications for research into vulnerability to stress or the interactions of genetic and environmental factors using stress or psychiatric disease-related genetic animal models.

Acknowledgements

This work was supported, in part, by Grants-in-Aid for "Academic Frontier Project for Private Universities (2007–2011) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; Research on the Risk of Chemical Substances from the Ministry of Health, Labour and Welfare, Japan; the Japan France Joint Health Research Program (Joint Project from Japan Society for the Promotion of Science); and an International Research Project Supported by the Meijo Asian Research Center.

References

[1] Abe H, Hidaka N, Kawagoe C, Odagiri K, Watanabe Y, Ikeda T. Prenatal psychological stress causes higher emotionality, depression-like behavior, and elevated activity in the hypothalamo-pituitary-adrenal axis. Neurosci Res 2007;59:145–51.
[2] Anisman H, Zaharia MD, Meaney MJ, Merali Z. Do early-life events permanently alter behavioral and hormonal responses to stressors? Int J Dev Neurosci 1998;16:149–64.
[3] Barbazanges A, Vallée M, Mayo W, Day J, Simon H, Le Moal M, et al. Early and later adoptions have different long-term effects on male rat offspring. J Neurosci 1996;16:7783–90.
[4] Beckett C, Maughan B, Rutter M, Castle J, Colvert E, Groothues C, et al. Do the effects of early severe deprivation on cognition persist into early adolescence? Findings from the English and Romanian adoptees study. Child Dev 2006;77(3):696–711.
[5] Birnbaum S, Gobeske KT, Auerbach J, Taylor JR, Arnsten AF. A role for norepinephrine in stress-induced cognitive deficits: α-1-adrenoceptor mediation in the prefrontal cortex. Biol Psychiatry 1999;46:1266–74.
[6] Cannon TD, van Erp TG, Bearden CE, Loewy R, Thompson P, Toga AW, et al. Early and late neurodevelopmental influences in the prodrome to schizophrenia: contributions of genes, environment, and their interactions. Schizophr Bull 2003;29(4):653–69.
[7] Charney DS. Psychobiological mechanisms of resilience and vulnerability: implications for successful adaptation to extreme stress. Am J Psychiatry 2004;161:195–216.
[8] Darnaudéry M, Koehl M, Barbazanges A, Cabib S, Le Moal M, Maccari S. Early and later adoptions differently modify mother–pup interactions. Behav Neurosci 2004;118:590–6.
[9] Enthoven L, de Kloet ER, Oitzl MS. Effects of maternal deprivation of CD1 mice on performance in the water maze and swim stress. Behav Brain Res 2008;187:195–9.
[10] Francis DD, Szegda K, Campbell G, Martin WD, Insel TR. Epigenetic sources of behavioral differences in mice. Nat Neurosci 2003;6(5):445–6.
[11] Friedman E, Berman M, Overstreet D. Swim test immobility in a genetic rat model of depression in modified by maternal environment: a cross-foster study. Dev Psychobiol 2006;48:169–77.
[12] Gomez-Serrano M, Tonelli L, Listwak S, Sternberg E, Riley AL. Effects of cross fostering on open-field behavior, acoustic startle, lipopolysaccharide-induced corticosterone release, and body weight in Lewis and Fischer rats. Behav Genet 2001;31:427–36.
[13] Heim C, Nemeroff CB. The impact of early adverse experiences on brain systems involved in the pathophysiology of anxiety and affective disorders. Biol Psychiatry 1999;46:1509–22.
[14] Juffer F, van Ijzendoorn MH. Behavior problems and mental health referrals of international adoptees: a meta-analysis. JAMA 2005;293:2501–15.
[15] Keyes MA, Sharma A, Elkins IJ, Iacono WG, McGue M. The mental health of US adolescents adopted in infancy. Arch Pediatr Adolesc Med 2008;162(5):419–25.
[16] Konno K, Matsumoto M, Togashi H, Yamaguchi T, Izumi T, Watanabe M, et al. Early postnatal stress affects the serotonergic function in the median raphe nuclei of adult rats. Brain Res 2007;1172:60–6.
[17] Lehmann J, Pryce CR, Jongen-Relo AL, Stohr T, Pothuizen HH, Feldon J. Comparison of maternal separation and early handling in terms of their neurobehavioral effects in aged rats. Neurobiol Aging 2002;23:457–66.
[18] Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 1987;92:180–5.
[19] Lysaker PH, Salyers MP. Anxiety symptoms in schizophrenia spectrum disorders: associations with social function, positive and negative symptoms, hope and trauma history. Acta Psychiatr Scand 2007;116(4):290–8.
[20] Maccari S, Morley-Fletcher S. Effects of prenatal restraint stress on the hypothalamus–pituitary–adrenal axis and related behavioural and neurobiological alterations. Psychoneuroendocrinology 2007;32(Suppl. 1):S10–5.
[21] Mamiya T, Kise M, Morikawa K. Ferulic acid attenuated cognitive deficits and increase in carbonyl proteins induced by buthionine-sulfoximine in mice. Neurosci Lett 2008;430:115–8.
[22] Matsumoto M, Higuchi K, Togashi H, Koseki H, Yamaguchi T, Kanno M. Early postnatal stress alters the 5-HTergic modulation to emotional stress at postadolescent periods of rats. Hippocampus 2005;15:775–81.
[23] Miyagawa H, Hasegawa M, Fukuta T, Amano M, Yamada K, Nabeshima T. Dissociation of impairment between spatial memory, and motor function and emotional behavior in aged rats. Behav Brain Res 1998;91:73–81.
[24] Mizoguchi K, Shoji H, Ikeda R, Tanaka Y, Tabira T. Persistent depressive state after chronic stress in rats is accompanied by HPA axis dysregulation and reduced prefrontal dopaminergic neurotransmission. Pharmacol Biochem Behav 2008;91(1):170–5.
[25] Mouri A, Noda Y, Hara H, Mizoguchi H, Tabira T, Nabeshima T. Oral vaccination with a viral vector containing Abeta cDNA attenuates age-related Abeta accumulation and memory deficits without causing inflammation in a mouse Alzheimer model. FASEB J 2007;21:2135–48.
[26] Nickman SL, Rosenfeld AA, Fine P, Macintyre JC, Pilowsky DJ, Howe RA, et al. Children in adoptive families: overview and update. J Am Acad Child Adolesc Psychiatry 2005;44(10):987–95.
[27] Noda Y, Yamada K, Furukawa H, Nabeshima T. Enhancement of immobility in a forced swimming test by subacute or repeated treatment with phencyclidine: a new model of schizophrenia. Br J Pharmacol 1995;116:2531–7.
[28] Nulman I, Rovet J, Greenbaum R, Loebstein M, Wolpin J, Pace-Asciak P, et al. Neurodevelopment of adopted children exposed in utero to cocaine: the Toronto Adoption Study. Clin Invest Med 2001;24(3):129–37.
[29] Pascual R, Zamora-León SP. Effects of neonatal maternal deprivation and postweaning environmental complexity on dendritic morphology of prefrontal pyramidal neurons in the rat. Acta Neurobiol Exp (Wars) 2007;67(4):471–9.
[30] Priebe K, Brake WG, Romeo RD, Sisti HM, Mueller A, McEwen BS, et al. Maternal influences on adult stress and anxiety-like behavior in C57BL/6J and BALB/CJ mice: a cross-fostering study. Dev Psychobiol 2005;47:398–407.
[31] Qiao H, Noda Y, Kamei H, Nagai T, Furukawa H, Miura H, et al. Clozapine, but not haloperidol, reverses social behavior deficit in mice during withdrawal from chronic phencyclidine treatment. Neuroreport 2001;12:11–5.
[32] Sharma AR, McGue M, Benson P. The emotional and behavioral adjustment of United States adopted adolescents, part II: age at placement. Child Youth Serv Rev 1996;18(1–2):101–14.
[33] Son GH, Geum D, Chung S, Kim EJ, Jo JH, Kim CM, et al. Maternal stress produces learning deficits associated with impairment of NMDA receptor-mediated synaptic plasticity. J Neurosci 2006;22–26:3309–18.
[34] Sullivan R, Wilson DA, Feldon J, Yee BK, Meyer U, Richter-Levin G, et al. The International Society for Developmental Psychobiology Annual Meeting Symposium: impact of early life experiences on brain and behavioral development. Dev Psychobiol 2006;48:583–602.
[35] Treit D, Fundytus M. Thigmotaxis as a test for anxiolytic activity in rats. Pharmacol Biochem Behav 1988;31:959–62.
[36] Vallée M, Mayo W, Dellu F, Le Moal M, Simon H, Maccari S. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. J Neurosci 1997;17:2626–36.
[37] Van der Veen R, Abrous DN, de Kloet ER, Piazza PV, Koehl M. Impact of intra- and interstrain cross-fostering on mouse maternal care. Genes Brain Behav 2008;7:184–92.
[38] Wierzbicki M. Psychological adjustment of adoptees: a meta-analysis. J Clin Child Psychol 1993;22(4):447–54.
[39] Wigger A, Loerscher P, Weissenbacher P, Holsboer F, Landgraf R. Cross-fostering and cross-breeding of HAB and LAB rats: a genetic rat model of anxiety. Behav Genet 2001;31:371–82.
[40] Yamada K, Iida R, Miyamoto Y, Saito K, Sekikawa K, Seishima M, et al. Neurobehavioral alterations in mice with a targeted deletion of the tumor necrosis factor-alpha gene: implications for emotional behavior. J Neuroimmunol 2000;111:131–8.
[41] Yamada K, Noda Y, Nakayama S, Komori Y, Sugihara H, Hasegawa H, et al. Role of nitric oxide in learning and memory and in monoamine metabolism in the rat brain. Br J Pharmacol 1995;115:852–8.

Hippocampus

The role of cyclophilin D in learning and memory

Journal:	*Hippocampus*
Manuscript ID:	HIPO-09-028.R1
Wiley - Manuscript type:	Research Article
Keywords:	Cyclophilin D , Cyclosporine A, mitochondrial membrane permeability transition , neurotransmission, learning and memory

Hippocampus

Title: The role of cyclophilin D in learning and memory

Akihiro Mouri[1,2], Yukihiro Noda[3], Shigeomi Shimizu[4,5], Yoshihide Tsujimoto[4], Toshitaka Nabeshima[1,6*]

1) Department of Chemical Pharmacology, Meijo University Graduate School of Pharmaceutical Sciences, Nagoya 468-8503, Japan.

2) Division of Scientific Affairs, Japanese Society of Pharmacopoeia, Tokyo 150-0002, Japan

3) Division of Clinical Sciences and Neuropsychopharmacology, Meijo University Graduate School of Pharmaceutical Sciences, Nagoya 468-8503, Japan.

4) Laboratory of Molecular Genetics, Department of Medical Genetics, Osaka University Medical School, Osaka 565-0871, Japan

5) Department of Pathological Cell Biology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo 113-8510, Japan

6) Japanese Drug Organization of Appropriate Use and Research, Nagoya 468-0069, Japan

Running title: The role of cyclophilin D in learning and memory

The number of Pages: 35, Figures: 8, Tables: 0

John Wiley & Sons

*Address correspondence to: Toshitaka Nabeshima

150 Yagotoyama, Tenpaku-ku, Nagoya 468-8503, Japan

Phone: 81-52-839-2735, FAX: 81-52-839-2738

e-mail: tnabeshi@ccmfs.meijo-u.ac.jp

Keywords: Cyclophilin D (Cyp D), Cyclosporine A (CsA), mitochondrial membrane permeability transition (MPT), neurotransmission, learning and memory, hippocampus

Hippocampus

Abstract

Cyclophilin D (Cyp D) is implicated cell death pathway and blockade of Cyp D could be a potent therapeutic strategy for degenerative disorders such as Alzheimer's disease, ischemia and multiple sclerosis. But, physiological role of Cyp D remains elusive. Here, we investigated the ability of learning and memory in several behavioral tasks in mice lacked Cyp D (Cyp $D^{-/-}$) and the relationship between ability of learning and memory and hippocampal architecture or neuronal transmission in Cyp $D^{-/-}$ mice. Cyp $D^{-/-}$ mice showed impairments of short-term memory in the Y-maze, object recognition memory in the novel-object recognition test, reference memory in the water maze test, and associative learning in the conditioned fear learning test. Hippocampal infusion of cyclosporine A (CsA), which binds to Cyp D, replicated the defect in hippocampus-dependent cognition observed in Cyp $D^{-/-}$ mice. The Cyp $D^{-/-}$ mice did not show histopathological abnormalities upon Nissl staining and GFAP immunostaining or irregular expression of neuronal and glial marker proteins on Western blotting. However, release of glutamate and acetylcholine was decreased from the hippocampus in response to high-potassium treatment in the Cyp $D^{-/-}$ mice than wild-type mice. These results suggest a physiological role for Cyp D in learning and memory via the regulation of neurotransmission. (204 words)

John Wiley & Sons

Introduction

Mitochondria are important in the control of both cell survival and cell death and the mitochondrial dysfunction is implicated in neurodegenerative disorders as well as in acute brain disease (Norenberg and Rao, 2007; Schinzel et al., 2005; Forte et al., 2008; Du et al., 2008). Dysregulation of mitochondrial membrane permeability transition (MPT) leads to apoptosis or necrosis (Norenberg and Rao, 2007). MPT is a regulated Ca^{2+}-dependent increase in the permeability of the mitochondrial membrane, which results in a loss of membrane potential, mitochondrial swelling, and rupture of the outer membrane (Zoratti and Szabò, 1996; Halestrap et al., 2002). MPT is proposed to occur after the opening of a channel termed the permeability transition pore and putatively composed of the voltage-dependent anion channel (VDAC) in the mitochondrial outer membrane, the adenine nucleotide translocase (ANT) in the inner membrane, and Cyclophilin-D (Cyp D) in the matrix (Crompton et al., 1998; Woodfield et al., 1998; Kokoszka et al., 2004). Although the involvement of VDAC and ANT in MPT is still controversial, experiments with Cyp D gene (*ppif*)-deficient mice indicate that Cyp D is involved in MPT, at least a cyclosporine-inhibitable form of MPT (Nakagawa et al., 2005; Bairns et al., 2005; Basso et al., 2005; Shinzel et al., 2005). Cyp D is a peptidylprolyl *cis*-trans isomerase thought to facilitate conformational change of putative targets such as ANT, to trigger MPT (Leung and Halestrap, 2008). Cyp D, encoded by peptidylprolyl *cis*-trans isomerase (*ppif*), Cyp D-deficient mice (Cyp $D^{-/-}$) are primarily protected from necrotic, caspase-independent cell death but not from caspase-dependent apoptosis (Nakagawa et al., 2005; Baines et al., 2005; Basso et al., 2005). Cyp D deficiency provides substantial protection from damage to both the heart and brain caused by ischemia/reperfusion (Nakagawa et al., 2005; Baines et al., 2005; Schinzel et al., 2005).

Recent studies reported that Cyp D deficiency protects from experimental autoimmune encephalomyelitis-induced axonal injury and motor dysfunctions as a model of multiple sclerosis (Forte et al., 2008) and amyloid-β-induced neuronal apoptosis in cultured neuron and impairments of cognitive function and plasticity in amyloid precursor protein transgenic mice as a model of Alzheimer`s disease (Du et al., 2008). In pharmacological approach, Cyclosporine A (CsA) , which binds to Cyp D to inhibit MPT (Halestrap and Davidson, 1990), and its analogues (N-mehyl-val^4-cyclosprin and FR901459) prevent neuronal degeneration in ischemia models (Matsumoto et al., 1999, Muramatsu et al., 2007). These results suggest that Cyp D and MPT is therapeutic target for these degenerative disorders.

Although, as a forementioned, a pathological role of Cyp D and MPT has been uncovered, their physiological role remains elusive. Mitochondria assist in maintaining Ca^{2+} homeostasis by sequestering and releasing Ca^{2+} (Nicholls and Budd, 2000; Bernardi, 1999). Synaptic mitochondria are synthesized in the cell body of neurons and transported to axons and dendrites (Morris and Hollenbeck, 1993; Kang et al., 2008). Mitochondria are present at high concentrations in presynpatic terminals (Shepherd and Harris, 1998; Rowland et al., 2000; Brown 2006). Neurotransmitter release is driven by an elevation of the Ca^{2+} concentration within the presynaptic terminal (Dodge and Rahamimoff, 1967, Long et al., 2008). Thus, some researchers have reported that mitochondria play a pivotal role in the release of neurotransmitters and short-term plasticity (Tang and Zucker, 1997; Billups and Forsythe, 2002; Lee et al., 2007; Kang et al., 2008). Under physiological conditions, Cyp D-dependent MPT has been suggested to be involved in Ca^{2+} buffering and, thus, to play an important role in learning and memory, and synaptic plasticity (Weeber et al., 2002; Levy et al., 2003). CsA impairs

long-term potentiation (LTP) and pre-pulse facilitation (Levy et al., 2003), and mice lacking VDAC isoforms show deficits in spatial and associative learning and synaptic plasticity (Weeber et al., 2002). Conversely, mice lacking Cyp D shows enhanced response in avoidance tests (Luvisetto et al., 2008) and normal synaptic plasticity and spatial memory in radial water maze test (Du et al., 2008). Further various behavioral experiments are needed for exploring the roles of CypD in learning and memory.

In the present study, we investigated the performance of several learning and memory tasks in mice lacking Cyp D and mice infused with CsA into the hippocampus, and found cognitive dysfunction.

Materials and Methods

Mice

Male C57BL/6J mice (7 weeks old) were obtained from Japan SLC (Shizuoka, Japan). Mice lacking Cyp D were described in Nakagawa et al. (2005). The homozygous mutant male mice (-/-; 3 months of age) and the littermate wild-type male mice (+/+; 3 months of age) were obtained by crossing F10 heterozygous Cyp D mutant mice (+/-) having a 99.99% pure C57BL/6J genetic background. The genotypes of mice were determined by PCR (Fig. 1A). The wild-type allele (553 base pairs, bp) was detected using as a forward primer, 5'-GCAGATCAAGCTCCCGACTG-3', and as a reverse primer, 5' -ACTTGGGAAGCCGAGGTG-3'. To detect the mutant allele (206 bp), a neomycin-specific reverse primer (5' -GCAGCGCATCGCCTTCTATC-3') was used in combination with the wild-type forward primer described above as described by Nakagawa et al. (2005). The animals were housed in plastic cages and kept in a regulated environment (24 ± 1°C, 50 ± 5% humidity), with a 12-hr light/dark cycle (lights on at 9:00 A.M.). Food and tap water were available ad libitum. All experiments were performed in accordance with the Guidelines for Animal Experiments of Meijo University. The procedures involving animals and their care were conducted in conformity with the international guidelines, Principles of Laboratory Animal Care (National Institutes of Health publication 85-23, revised 1985).

Surgery

Under anesthesia (pentobarbital 40 mg/kg, i.p.), C57BL/6J mice were placed in a stereotaxic apparatus and bilaterally implanted with a guide cannula (12 mm, 0.4 mm in inner diameter, 0.5 mm in outer diameter; Eicom) in the hippocampus (-2.2 mm