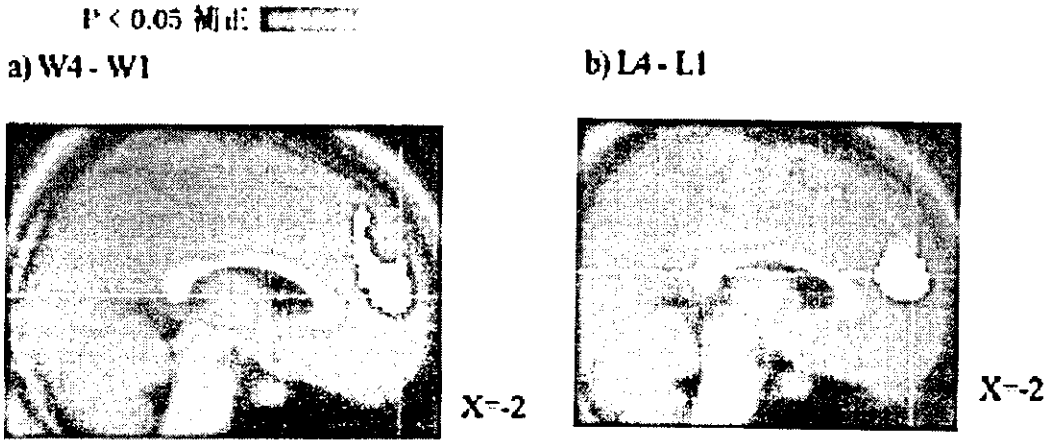
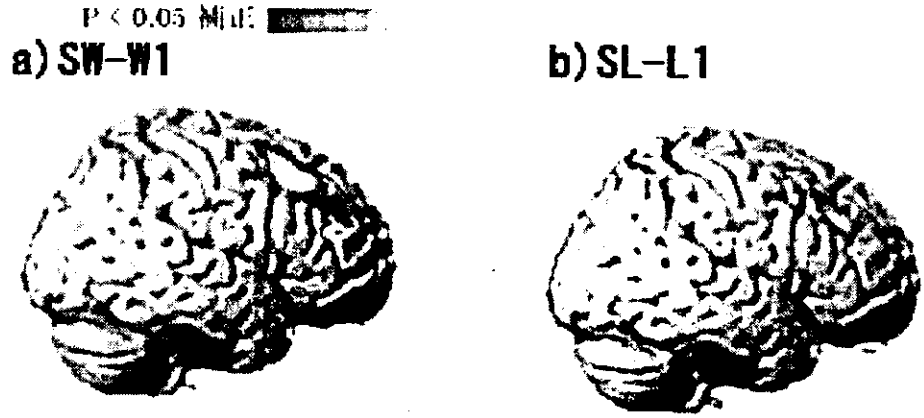
図1　報酬や罰が連続する文脈に関わる脳活動



a) 4連勝目（W4)が通常の1勝(W1)に比較して有意な脳活動の上昇を示した領域
b) 4連敗目（L4)が通常の1敗（L1)に比較して有意な脳活動の上昇を示した領域
いずれも多重比較補正後（$p < 0.05$）の領域を示す。共通して前頭前野内側面および帯状回前部で有意な脳活動が認められており、この領域が報酬・罰の性質が連続する文脈に関わる脳活動であると考えられる。またこの領域は、被験者の内省報告のスコアとも活動が相関しており、主観的情動体験とも関わりのある領域と考えられた。

図2　報酬や罰の性質が切り替わる文脈に関わる脳活動



a)　4連敗後の1勝（SW)が通常の1勝(W1)に比較して有意な脳活動の上昇を示した領域
b)　4連勝後の1敗（SL)が通常の1敗（L1)に比較して有意な脳活動の上昇を示した領域
いずれも多重比較補正後（$p < 0.05$）の領域を示す。共通して右前頭前野背外側部で有意な脳活動が認められており、この領域が報酬・罰の性質が切り替わる文脈に関わる脳活動であると考えられる。

Ⅲ．研究成果の刊行に関する一覧表

雑誌（代表的なもののみ）

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Akitsuki Y, Sugiura M, Watanabe J, Yamashita K, Sassa Y, Awata S, Matsuoka H, Matsue Y, Fukuda H, Kawashima R	Context-dependent cortical activation in response to financial reward and penalty: An event-related fMRI study	NeuroImage	19	1674-1685	2003
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Context-dependent cortical activation in response to financial reward and penalty: an event-related fMRI study

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Abstract

An event-related fMRI technique was used to assess neural responses to financial reward and penalty during a simple gambling task. We attempted to determine whether brain activities are dependent on the unique context of an event sequence. Thirty-six healthy volunteers participated in the study. The task was to guess the color of the suit of a card on each trial and to respond by pressing a button. Every correct response ("win") and incorrect response ("loss") was associated with financial reward and penalty, respectively. The magnitude of reward or penalty in each trial did not change; however, the subjects' self-reported emotional arousal was significantly higher for the events of "the fourth win of four wins in a row" and "the fourth loss of four losses in a row." We also found that the bilateral anterior cingulate and medial prefrontal cortices were specifically activated when the subjects experienced "the fourth win of four wins in a row" and "the fourth loss of four losses in a row. "When the subjects experienced "a win following four losses in a row" or "a loss following four wins in a row, "the right dorsolateral prefrontal cortex was specifically activated. Our data indicate that there exist brain activities associated with the event-sequence context in which abstract reward or penalty is received. These context-dependent activities appear to be crucial for adapting oneself to new circumstances and may account for clinical symptoms of various mental illnesses in which dysfunction of these regions has been reported.

Introduction

Receiving rewards or penalties is not a simply passive behavior. Animals should predict potential rewards/penalties, distinguish their nature, and respond appropriately by changing their attentional or arousal level for successful adaptive behavior. Single-unit studies of nonhuman primates revealed the brain structures that are related to rewards and penalties. In the monkey, midbrain dopamine neurons were reported to be activated by the occurrence of rewards after their presentation and visual or auditory stimuli that predict rewards (Schultz, 1986; Romo and Schultz, 1990; Schultz and Romo, 1990; Ljungberg et al., 1992). Dopamine neurons also encode an error in the temporal prediction of rewards; the dopamine activity is enhanced by surprising rewards and it is depressed by the omission of predicted rewards (Ljungberg et al., 1992; Mirenowicz and Schultz, 1994). Other neurons in the ventral striatum (Apicella et al., 1991; Shidara et al., 1998) and orbitofrontal cortex (Niki et al., 1972) also respond to the delivery of

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rewards. Furthermore, neurons within the orbitofronal cortex not only discriminate rewards and penalties (Thorpe et al., 1983), but also respond to reward expectancy (Tremblay and Schultz, 1999, 2000). Reward expectation neurons are also found in the striatum (Shidara et al., 1998; Schlutz et al., 1992). Neurons in the lateral prefrontal cortex were reported to be sensitive to the presence or absence of expected rewards (Watanabe, 1990, 1992) and play an important role in coding the discrepancy between the expectancy of a specific reward and the response outcome (Watanabe, 1996).

On the other hand, imaging studies revealed that human brain activities are associated with the cognition of abstract rewards and penalties. In particular, the "gambling task" has been recently used as a laboratory model for investigating brain activities in response to abstract types of reward and punishment (e.g., monetary reward or penalty) in normal subjects. A previous positron emission tomography (PET) study revealed that the human orbitofrontal cortex can be activated with monetary reward (Thut et al., 1997). A functional magnetic resonance imaging (fMRI) study supports this finding, demonstrating that an increase in the activity of the medial orbitofrontal cortex is related to the subjects' receipt of monetary reward and deactivation following punishments (O'Doherty et al., 2001). The authors recorded the converse pattern of activation in the lateral orbitofrontal cortex (activation following punishments, deactivation following rewards). Moreover, in these areas, the authors found a correlation between the magnitude of brain activation and that of rewards and punishments received.

However, neural responses related to human reward systems under different psychological contexts are studied less extensively. On this issue, some groups focused on the context of anticipation of abstract rewards and punishments. They demonstrated that whereas anticipation of increasing rewards elicited nucleus accumbens activation (Breiter et al., 2001; Knutson et al, 2001b), the anticipation of increasing punishment did not activate the nucleus accumbens (Knutson et al., 2001a). Nucleus accumbens activation correlated with individual differences in self-reported happiness elicited by the reward cues (Knutson et al., 2001a). On the other hand, Elliott and colleagues (2000) focused on the psychological context of a winning or losing streak. They demonstrated that brain activities in the bilateral globus pallidus, thalamus, and subgenual cingulate are associated with rewards in the context of winning streak. They also showed that responses to both rewards occurring as part of a winning streak and penalties as part of a losing streak are observed in the caudate, insula, and ventral prefrontal cortex. This is consistent with the findings that the medial orbitofrontal and ventromedial prefrontal cortices are involved in the representation of rewards and risk (Bechara et al., 1994, 1996, 1997; Rolls et al., 1994).

Taking these results into account, a prior emotional experience with streaks of rewards/penalties appears to shape the subjective "feeling" of an incoming expected reward/penalty or an unexpected one for that matter. Moreover, there seems to be dissociable neural responses that are dependent on the psychological context in which abstract rewards and penalties are received in the human brain. Therefore, based on the study by Elliott et al., we would like to determine the relationship between subjective feelings and the context of winning/losing streaks and how these context are expressed in the human brain. In this experiment, an event-related fMRI technique was used to assess neural responses to financial reward and penalty during a gambling task whose paradigm was based on the study by Elliott and Critchely (Critchley et al., 2000). Our aim is to determine whether subjective feelings and brain activities are dependent on the unique context of an event sequence, that is, four wins in a row, four losses in a row, a win following four losses in a row, or a loss following four wins in a row. On the basis of previous studies, we hypothesize that:

1. The medial prefrontal cortex is activated by risk-tasking and emotionally salient events, such as the context of a winning or losing streak.
2. The lateral prefrontal cortex is activated by the discrepancy between the expectancy and the outcome of rewards/penalties, such as the context of switching from a winning or losing streak to the opposite event.

Methods

Subjects

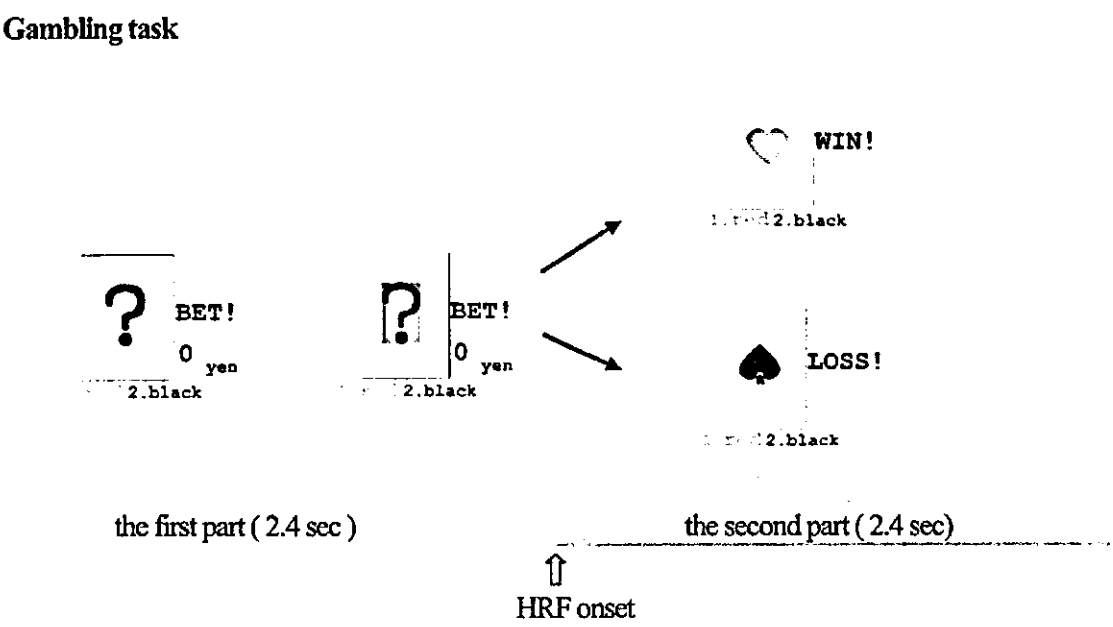
Thirty-six healthy volunteers (19 men and 17 women), whose mean age was 20.8 years (SD = 2.98), participated in this study. All were native Japanese speakers and right-handed, as assessed by the Edinburgh Handedness Inventory. Subjects with a history of psychiatric, neurological, or other serious physical illnesses; drug or alcohol abuse; or second-degree relatives with a history of major psychiatric disorders were excluded.

Approval to carry out this experiment was obtained from Ethics Committee of Tohoku Fukushi University. In accordance with the Helsinki Declaration of Human Rights (1975), written informed consent was obtained from all participants after complete and detailed description of the study to the subjects.

Experimental paradigm

Brain activity associated with the psychological context of reward/penalty in a simple gambling task was examined, using an event-related fMRI technique. The experimental paradigm we used here was based on the previous studies done by Elliott and Critchley (Elliott et al., 2000; Critchely et al., 2000).

Subjects were presented with cards as visual stimuli on a



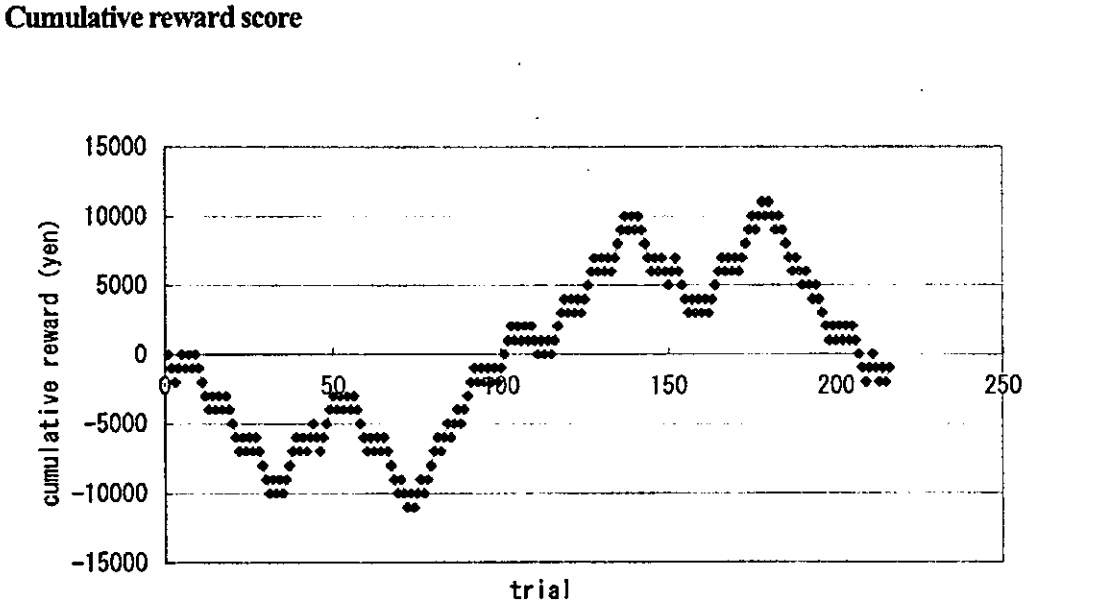


Fig. 1. Gambling task (top). During the first part, subjects were instructed to guess the color of the suit and respond by pressing a button. (red, button 1; black, button 2). During the second part, the correct color and a "win!" or "loss!" message were presented. HRF, hemodynamic response function. Cumulative reward score (bottom). Because the outcome feedback ("win" or "loss") sequence was predetermined regardless of the subjects' actual choices, the cumulative reward score was also predetermined as shown here.

small screen on a head coil. The task was to guess the color of the suit (red or black) of a card on each trial and respond by pressing a button. (Fig. 1) Each trial consists of two parts and each part lasted 2.4 s, so that the duration of the entire trial was 4.8 s. In the first part, a card was presented for 2.4 s, and during this period, subjects were instructed to press button 1 if they thought the suit color would be red or press button 2, if black. In the first part, the cumulative "reward" score was also presented. Subjects began this task with a 0 yen "stake." For the subsequent 2.4 s (the second part), the correct color and a "win!" or "loss!" message were presented. Every correct response (win) in the second part was associated with an increment of 1000 yen in the cumulative reward score for the subsequent first part of the next

trial, whereas every incorrect response (loss) was associated with a decrement of 1000 yen in the score. In fact, this outcome feedback (win or loss) sequence was predetermined according to a pseudorandom sequence, regardless of the subjects' actual choices. Therefore, the cumulative reward score was also predetermined (Fig. 1).

To examine the neural response related to the context in which rewards and penalties are experienced in this task, artificial repetition of win or loss was designed, that is, "four wins in a row" and "four losses in a row." There were 215 trials in each run. The total number of win versus loss, "winning four trials in a row" versus "losing four trials in a row" and "a win subsequent to losing four trials in a row" versus "a loss subsequent to winning four trials in a row" was counterbalanced across the task.

Immediately after the fMRI measurements, the subjects were asked to indicate how happy or unhappy they found themselves at winning or losing events during the task performance using an 11-point rating scale (i.e., "5" indicates "happiest," "0" indicates "neutral," and "−5" indexes "unhappiest").

fMRI measurement

T1-weighted structural images were acquired for each volunteer, using a 1.5-T Siemens Vision plus scanner (Siemens Magnetron Vision, Erlanger, Germany; repetition time 9.7 ms, echo time 4 ms, flip angle 12°, FOV = 250 mm, pixel size 1.25 × 0.98 mm, matrix 175 × 256). To measure neural responses, gradient echo, echoplanar T2*-weighted images with blood oxygenation level-dependent (BOLD) contrast were acquired (repetition time 4000 ms, echo time 60 ms, flip angle 90°, FOV = 256 × 256 × 256 mm^3, voxel size 4 × 4 × 3 mm^3, matrix 64 × 64, slice thickness 3mm, interslice gap 0.99 mm). Thirty-four axial slices were oriented over the whole brain.

To allow for T1 equilibration effects, two preliminary scans were acquired and subsequently discarded. Thereafter, scans were acquired continuously every 4.0 s, so each block of five behavioral trials corresponded to six scans. This temporal asynchrony is significant for avoiding a systematic bias in sampling over peristimulus time.

Data analysis

Data were analyzed on a work station (Sun Microsystems), using MATLAB (Mathworks Inc., Natick, MA) and statistical parametric mapping (SPM99; Wellcome Department of Cognitive Neurology, London, UK). The initial two scans were discarded for the nonequilibrium state of magnetization.

Before statistical analysis, a series of spatial transformation stages were required. First, slice time adjustment was performed to correct for differences in acquisition time among 34 slices of each scan. Second, to correct for artifacts caused by small head movements, images from each subject

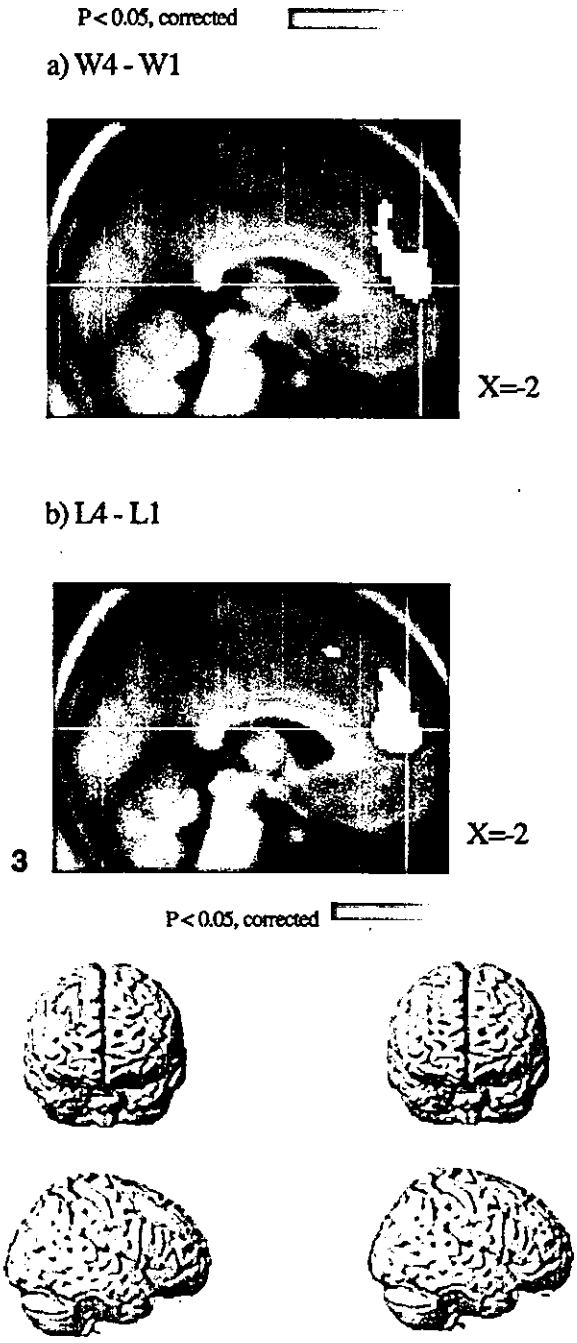


Fig. 3. Brain areas that were significantly activated when subjects experience four wins in a row and four losses in a row. Depicted here are areas of the brain that yielded significantly higher activity in W4 than in W1 (a), and in L4 than in L1 (b) and rendered onto the mean anatomically standardized T1 image of all subjects. Both activation were significant at $P < 0.05$ (corrected).

Fig. 4. Brain areas that were significantly activated when subjects experience a win following four losses in a row and a loss following four wins in a row. Depicted here are areas of the brain that yielded significantly higher activity in SW than in W1 (a) and in SL than in L1 (b). Both activations were significant at $P < 0.05$ (corrected). Frontal views are shown in the top panel and lateral views from the right are shown in the lower panel. SW, sudden win; SL, sudden loss.

were realigned to the first image and resliced using a sinc interpolation. Third, a mean image created from the realigned volumes was coregistered with the structural T1 volume and the structural volumes were spatially normalized to a standard template in the space of Talairach and Tournoux (1988) using nonlinear basis functions. Fourth, the derived spatial transformation was applied to the realigned T2* volumes, which were then spatially smoothed with a three-dimensional isotropic Gaussian kernel (12 mm full-width at half-maximum). This improves the signal-to-noise ratio and accommodates for residual variations in functional neuroanatomy that usually persist between subjects after spatial normalization.

A key aspect of rewarding situation is how reward (or penalty)-related responses are modulated by the psychological context. We, therefore, hypothesized that neural responses depend on the context of winning or losing streak and presumed the following 10 conditions for each subject:

1. W1: the win other than W2, W3, W4, and SW
2. W2: the second win of two wins in a row
3. W3: the third win of four wins in a row
4. W4: the fourth win of four wins in a row
5. SW: a win following four losses in a row; SW stands for sudden win
6. L1: the loss other than L2, L3, L4, and SL
7. L2: the second loss of two losses in a row
8. L3: the third loss of three losses in a row
9. L4: the fourth loss of four losses in a row
10. SL: a loss following four wins in a row; SL stands for sudden loss

For both W4 and SW, which were designed as a special context, a subtraction method was used to contrast the brain activity associated with W1 that seems less meaningful or less exciting in terms of psychological context. On the other hand, for both L4 and SL, a subtraction method was used to contrast the brain activity associated with L1. We also compared the brain activities between W1 and L1, W4 and L4, and all wins (including W1, W2, W3, W4, and SW) and all losses (including L1, L2, L3, L4, and SL).

In addition, we performed the correlation analysis between the subjects' arousal and the fMRI signals. In this analysis, neural activities were examined for parametric modulation by the scores of arousal rating scale.

fMRI data were analyzed using the general linear model. For the statistical analysis on the intrasubject basis, the time series of images were correlated with the SPM99 built-in "Canonical HRF" that approximates activation patterns. The onset of the expected response was positioned at the beginning of the second part of each trial (i.e., the point at which the correct color of suit and a "win!" or "loss!" message were presented (see Fig. 1). Condition effects at each voxel were estimated according to this general linear model and regionally specific effects were compared using linear contrasts. Global changes were adjusted by proportional scaling and low-frequency confounding effects were removed using an appropriate high-pass filter. Voxel values for each contrast yielded a statistical parametric map of the t statistic (SPM t), which was subsequently transformed to the unit normal distribution (SPM Z).

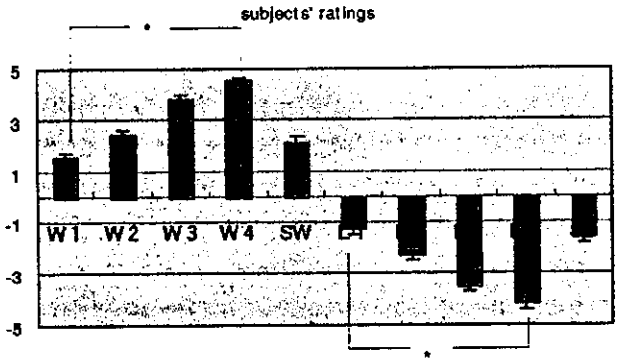


Fig. 2. Mean subjects' ratings. Mean subjects' ratings of emotional experience in each winning and losing event. Each subject rated each event individually (W1, W2, W3, W4, SW, L1, L2, L3, L4, and SL). SW stands for sudden win. SL stands for sudden loss. Two way ANOVA and post hoc Tukey's tests were performed. Subjects' ratings showed significant differences in W1 versus W4 and L1 versus L4 ($P < 0.000001$), but not in W1 versus SW and L1 versus SW.

Intersubject maps were then produced by performing one-sample t tests to identify voxels that survive a voxel-wise multiple comparison ($P < 0.05$, corrected). At this step, we used masking procedures: The resulting SPM{T} of W4 − W1, L4 − L1, SW − W1, and SL − L1 were masked with the statistically thresholded ($P < 0.05$ corrected) mask of W4, L4, SW, and SL, respectively.

Finally, the resulting activation maps were created and displayed by projection onto the mean anatomically standardized T1 image of all subjects to identify cortical structures.

Results

Subjects' ratings

Two-way ANOVA (analysis of variance) and post hoc Tukey's tests were performed. Examination of the subjects' self-reported ratings indicated that W4 was a significantly more pleasant/happier event than W1 (mean ± SD, W1, 1.58 ± 0.87; W4, 4.5 ± 0.78, $P < 0.000001$), and L4 was a significantly more unpleasant/unhappier event than L1 (mean ± SD, L1, −1.28 ± 1.03; L4, −4.17 ± 1.36, $P < 0.000001$) (Fig. 2).

However, there was no significant difference between W1 and SW (mean ± SD, L1, 1.58 ± 0.87; SW, 2.11 ± 1.58, $P > 0.05$) or between L1 and SL (mean ± SD, L1, −1.28 ± 1.03; SL, −1.58 ± 1.40, $P > 0.05$).

Table 1
Brain activities for four wins in a row and four losses in a row

	L/R	Region	Number of voxels (k)	Talairach coordinates (mm)			T score
				x	y	z	
W4 − W1							
	R	Medial aspect of superior frontal gyrus	81[a]	8	22	58	8.11
	R	Medial aspect of superior frontal gyrus		12	58	22	7.16
	L	Medial aspect of superior frontal gyrus	908[b]	−2	58	10	8.21
	L	Medial aspect of superior frontal gyrus		−2	40	46	6.85
L4 − L1							
	R	Medial aspect of superior frontal gyrus	1229	8	56	22	8.78
	R	Medial aspect of superior frontal gyrus	32	10	18	60	7.7
	L	Medial aspect of superior frontal gyrus	111[c]	−8	22	58	6.55
	L	Medial aspect of superior frontal gyrus		−4	50	20	8.35
	R	Middle temporal gyrus	22	60	−12	−12	5.29

Note. Maximally activated voxels in brain areas where significantly higher activities were found in W4 than in W1 and in L4 than in L1 ($P < 0.05$, corrected). Stereotaxic coordinates are derived from the human atlas of Talairach and Tournoux (1998) and refer to medial–lateral position (x) relative to the midline (positive = right), anterior–posterior position (y) relative to the anterior commissure (positive = anterior), and superior–inferior position (z) relative to the commissural line (positive = superior). L, left; R, right.

[a] This amount is the total number of activated voxels for the right medial aspect of the superior frontal gyrus in W4 − W1.

[b] This amount is the total number of activated voxels for the left medial aspect of the superior frontal gyrus in W4 − W1.

[c] This amount is the total number of activated voxels for the left medial aspect of the superior frontal gyrus in L4 − L1.

fMRI data

Figure 3a and Table 1 show the significantly activated regions in the subtraction analysis of W4 − W1. In the event of the fourth win of four wins in a row (W4), significant activation was observed in the medial aspect of the superior frontal gyrus and the anterior cingulate gyrus, in comparison with the W1 event. There were two activation peaks in the cluster of the right superior frontal gyrus and in that of the left superior frontal gyrus.

Figure 3b and Table 1 illustrate the significantly activated regions in the subtraction analysis of L4 − L1. In the event of the fourth loss of four losses in a row (W4), significant activation was found in the medial aspect of the frontal gyrus, the anterior cingulate gyrus, and the right middle temporal gyrus. There were two activation peaks in the cluster of the left medial aspect of the superior frontal gyrus. The activation pattern of W4 − W1 highly overlapped with that of L4 − L1 (Figs. 3a and b).

Table 2 shows the significantly activated regions in the subtraction analysis of W1 − L1. In the event of W1, significant activation was observed in the bilateral ventral striatum, bilateral lingual gyrus, bilateral inferior temporal gyrus, and bilateral posterior lobe of the cerebellum, compared with the L1 event. However, no significant activation was found in L1 − W1, W4 − L4, and L4 − W4.

Table 3 shows the comparison between neural activation for all wins and that for all losses. In the events of win, the bilateral ventral striatum, bilateral posterior lobe of the cerebellum, and left lateral occipital cortex were significantly activated in comparison with the events of loss. No significant activation was found in the subtraction analysis of all wins — all losses.

Table 4 shows the brain regions that correlate with the subjects' arousal. The medial aspect of the superior frontal gyrus and the anterior cingulate gyrus significantly correlate with the subjects' arousal in the events of both win and loss. This activation pattern appears to be very similar to those of W4 − W1 and that of L4 − L1 (Figs. 3a, b).

Figure 4a and Table 5 show the significantly activated regions in the subtraction analysis of SW − L1. In the event of a win after four losses in a row (SW), significant activation was observed in the right middle frontal gyrus, in comparison with the W1 event. There were two activation peaks in the cluster of the right middle frontal gyrus.

Figure 4b and Table 5 illustrate the significantly activated regions in the subtraction analysis of SL − L1. Compared with L1, in the event of a loss after four wins in a row (SL), significant activation was found in the right middle frontal gyrus. There were two activation peaks in the cluster of the right middle frontal gyrus. The activation pattern of SW versus W1 appears to be very similar to that of SL versus L1. (Figs. 4a and b)

Discussion

In this study, using a simple gambling task, we demonstrated brain activities associated with the event-sequence context in which abstract reward or penalty is received.

The most significant findings in our study are as follows:

1. The magnitude of each reward or penalty was the same across the whole events; however, psychological data demonstrated that subjective emotional experiences are different because of the context in which

Table 2
Brain activities for W1 versus L1 and W4 versus L4

	L/R	Region	Number of voxels (*k*)	Talairach coordinates (mm)			*T* score
				x	*y*	*z*	
W1 − L1							
	R	Ventral striatum	400	24	0	−8	8.67
	L	Ventral striatum	88	−26	−2	−12	8.61
	R	Lingual gyrus	10,028[a]	16	−68	−12	9.47
	L	Lingual gyrus		−16	−86	−2	10.63
	R	Inferior temporal gyrus		40	−78	−12	8.99
	L	Inferior temporal gyrus		−42	−62	−12	9.88
	R	Posterior lobe of cerebellum		−10	−72	−20	9.45
	L	Posterior lobe of cerebellum		−34	−64	−32	8.52
L1 − W1							
		NS					
W4 − L4							
		NS					
L4 − W4							
		NS					

Note. Maximally activated voxels in brain areas where significantly higher activities were found in W1 than in L1 ($P < 0.05$, corrected). No significant activation was found in L1 − W1, W4 − L4, and L4 − W4. NS, not significant.

[a] This amount is the total number of activated voxels for the bilateral lingual gyrus, inferior temporal gyrus, and posterior lobe of the cerebellum in W4 − L1.

reward or penalty is received, that is, the sequence of each reward/penalty-related event (i.e., four wins in a row, four losses in a row, a win following four losses in a row, and a loss following four wins in a row).

2. Depending on the nature of the unique context (i.e., repetition of win or loss vs. switching from a winning or losing streak to the opposite event), specific brain regions were activated, unrelated to the nature of event (i.e., reward-related vs. penalty-related).
   a. The medial aspect of the prefrontal cortex and the anterior cingulate cortex showed significant activations both in a series of reward-related events and in a series of penalty-related events. In these brain regions, there were significant correlations between fMRI signals and the subjects' arousal.
   b. The right dorsolateral prefrontal cortex showed significant activation in switching events, such as a reward-related event following a series of penalty-related event and a penalty-related event following a series of reward-related event.

We highlight the finding that the overlapping activation of brain regions was induced in a specific context, whether the event was reward-related or penalty-related. This means that information regarding abstract rewards and penalties is represented as a certain context in the human brain independent of its nature (i.e., reward-related or penalty-related). Then, what is the meaning of this context-dependent brain activity? Why is a specific brain region activated in the unique context, even if the magnitude of each reward or penalty is the same?

Table 3
Brain acitivities for all wins versus all losses

	L/R	Region	Number of voxels (*k*)	Talairach coordinates (mm)			*T* score
				x	*y*	*z*	
all W — all L							
	R	Ventral striatum	26	26	2	−10	6.88
	L	Ventral striatum	64	−24	4	−12	7.51
	R	Posterior lobe of cerebellum	2426[a]	38	−76	−28	8.69
	L	Posterior lobe of cerebellum		−28	−78	−20	8.15
	L	Lateral occipital cortex		−22	−92	−6	7.86
all L — all W							
		NS					

Note. Maximally activated voxels in brain areas where significantly higher activities were found in all W than in all L ($P < 0.05$, corrected). No significant activation was found in all L — all W. NS, not significant. all W, all wins including W1, W2, W3, W4, and SW. all L, all losses including L1, L2, L3, L4, and SL.

[a] This amount is the total number of activated voxels for the bilateral posterior lobe of the cerebellum and the lateral occipital cortex in all W — all L.

Table 4
Brain activities that correlate with the subjects' arousal

	L/R	Region	Number of voxels (*k*)	Talairach coordinates (mm)			*T* score
				x	*y*	*z*	
Wm							
	L	Medial aspect of superior frontal gyrus	1975[a]	−8	58	26	10.57
	R	Medial aspect of superior frontal gyrus		4	56	24	9.05
	L	Medial aspect of superior frontal gyrus		−4	46	10	8.53
	L	Medial aspect of superior frontal gyrus	92	−6	20	60	6.90
		Posterior cingulate gyrus	52	0	−24	38	6.51
Lm							
	R	Medial aspect of superior frontal gyrus	2528	4	52	24	9.76
	R	Middle temporal gyrus	3	58	−14	−22	5.06
	L	Middle temporal gyrus	25	−58	−14	−20	5.70
		Vermis	2	6	−62	−6	5.02

Note. Maximally activated voxels in brain areas that significantly correlated with the subjects' arousal ($P < 0.05$, corrected). Wm, modulation by the subjects' arousal in the events of win. Lm, modulation by the subjects' arousal in the events of loss.

[a] This amount is the total number of activated voxels for the bilateral medial aspect of superior frontal gyrus in Wm.

Medial aspect of prefrontal cortex

In our study, the maximum activation during a series of reward-related event and a series of penalty-related event (i.e., four wins in a row and four losses in a row) was localized to the cingulate sulcus, the anatomical border between the anterior cingulate (Brodmann's area 32), and the medial prefrontal cortex (Brodmann's area 9). Studies on cytoarchitectonic characteristics (Vogt et al., 1995) and laminar characteristics (Barbas and Pandya, 1989) of these two areas suggest that a rigid distinction between this portion of the anterior cingulate cortex and the medial prefrontal cortex is misleading. Given that the observed activation extended into both the anterior cingulate and the medial prefrontal cortex, this most likely reflects processes characteristic of these two regions.

Activation of the medial prefrontal cortex observed in our study appears to be involved in the emotionally salient context. Results of other imaging studies are consistent with our data. The medial prefrontal cortex is an area that was found to be activated in previous PET studies of emotion (George et al., 1995; Lane et al., 1997c). Since the medial prefrontal cortex appears to participate in aspects of emotion unrelated to its type, valence, or method of induction (Lane et al., 1997b; Reiman et al., 1997), it may be involved in the arousal component of emotional response as opposed to valence appraisal (Lang and Greenwald, 1988).

Several studies showed that the anterior cingulate cortex is related to pain (Peyron et al., 1999; Rainville et al., 1997), performance monitoring (Shima and Tanji, 1998), error detection (Carter et al., 1998), conflict monitoring (Botvinick et al., 1999), and response selection (Turken and Swick, 1999). The activation we observed in our study when subjects experience four wins in a row or four losses in a row, however, is more anterior to the foci reported in these studies. Our interpretation of the data is that the activation of the anterior cingulate cortex is related to emotional processing and subjective experience of emotion (Lane et al., 1997a). Some lesion studies support this idea. Ventromedial prefrontal lesions are associated with the ab-

Table 5
Brain activities for a win following four losses in a row and a loss following four wins in a row

	L/R	Region	Number of voxels (*k*)	Talairach coordinates (mm)			*T* score
				x	*y*	*z*	
SW − W1							
	R	Middle frontal gyrus	850[a]	34	22	52	7.52
	R	Middle frontal gyrus		26	42	38	5.98
SL − L1							
	R	Middle frontal gyrus	317[b]	36	30	36	5.38
	R	Middle frontal gyrus		26	16	54	4.94

Note. Maximally activated voxels in brain areas where significantly higher activities were found in SW than in W1 and in SL than in L1 ($P < 0.05$, corrected).

[a] This amount is the total number of activated voxels for the right middle frontal gyrus in SW − W1.

[b] This amount is the total number of activated voxels for the right middle frontal gyrus in SL − L1.

sence of autonomic response to positively as well as negatively charged pictures (Damasio et al., 1990). Ventral prefrontal lesions are related to changes in patients' ability to feel emotions compared with their premorbid state (Homak et al., 1996). This is consistent with Papez' suggestion (Papez, 1937) that this region is "the seat of emotional experience."

A study on single neuronal signal in the anterior cingulate cortex of monkeys demonstrated responses that progressively changed strength with reward expectancy (Shidara and Richmond, 2002). The authors hypothesized that the anterior cingulate cortex activity reflects or perhaps regulates the degree of motivation and/or reward expectancy as the multistage task progresses. A recent study of event-related brain potentials (ERPs) in human participants performing a monetary gambling task also suggests that the anterior cingulate cortex activity is involved in assessing the motivational impact of the outcome events (Gehring and Wilbughby, 2002). Taking these data into account, the activations of the anterior cingulate cortex in our study seem to be sequential-context-dependent, reflecting the strength of subjects' reward expectancy (and/or motivation) or premonition of penalty.

Lateral prefrontal cortex

On the other hand, the right dorsolateral prefrontal cortex appears to represent a different context in the human brain. Many studies indicate that the right dorsolateral prefrontal cortex is activated during the execution of the Wisconsin Card Sorting Test (WCST; Milner, 1963, 1971; Berman et al., 1995; Nagahama et al., 1996; Meyer-Lindenberg et al., 2002). The WCST is the standard task paradigm for detecting human frontal lobe dysfunction. The WCST has been used to assess the ability to shift a cognitive set from one perceptual attribute of a complex visual stimulus to another (i.e., set-shifting ability). For example, after a prescribed number of successive correct responses to one dimension (e.g., color), the previously irrelevant dimension (i.e., shape) becomes relevant and the subject must shift the sorting criterion to the other one.

In our data, the right dorsolateral prefrontal cortex showed significant activation in the event of switching between reward and penalty, such as "a win following four losses in a row" and "a loss following four wins in a row." Essentially, both in our study and in the WCST, subjects are challenged on switching events. For example, a succession of wins through a sequence of trials has the effect of promoting the development of an attentional and/or emotional bias toward the reward-related event. This bias is then challenged on a loss that requires the subject's attention and/or emotion to be directed away form reward-related event toward the newly penalty-related event and vice versa.

Thus, taking these data into account, the activation of the right dorsolateral prefrontal cortex may depend on the context in which the nature of event is switched from one to another. In other words, this region may be a critical area in higher-order shifting of attention and/or emotion between different conceptual categories of abstract values. This proposal is consistent with the result in previous studies that the right dorsolateral prefrontal cortex is associated with unpredictability and surprise violations of the learned association (Fletcher et al., 2001; Zeki and Marini, 1998; Fink et al., 1999). This is also consistent with the findings of single-unit studies of the monkey; that is, neurons in the lateral prefrontal cortex are sensitive to the presence or absence of an expected reward in delayed reaction time tasks (Watanabe, 1990, 1992). Thus, the dorsolateral prefrontal cortex may be the key region for monitoring an ongoing outcome and essential for adaptive behavior.

Other related issues

Our study was based on the previous study performed by Elliott et al. (2000); however, our results are different from theirs. Elliott et al. examined the differential responses to wins occurring as a part of a winning streak and the differential responses to losses occurring as a part of a losing streak. This is conceptually similar to our results of the W4 − W1 and L4 − L1 subtractions, but our results diverged from those of Elliot's. A possible explanation for this divergence is the method of analysis and the design of our experiment (i.e., the sequence of win or loss events), which are different from theirs. Elliott and colleagues focused on the brain activities that were modulated by "how fast the reward level is changing." Moreover, they reported that there were no neural responses associated with a main effect of winning or losing streak. Thus, the regions they detected to be responsive to a winning or losing streak were activated only in contexts in which there was a congruence between the rate of change and the reward level. On the other hand, we took a different approach to the analysis, using a more simple analytical method: we focused on brain activities that were modulated by how each win or loss event was repeated, regardless of the current reward level. Note that, to minimize the effect of this reward level, we designed the win/loss sequence such that the list of scores at which each W4 (i.e., four wins in a row) occurs is the same as that of scores at which each L4 (i.e., four losses in a row) occurs across the trial. This is why Fig. 1 shows point symmetry. Furthermore, we set the wining and losing streaks as four wins in a row and four losses in a row, respectively. This fixed design is more likely to produce subjects' feelings over the winning/losing streaks and enabled us to more efficiently detect the brain activities associated with these streaks. In fact, increased neuronal activities were observed at the anterior cingulate in a single-unit animal study in which the sequence of reward/no-reward events were fixed (Shidara and Richmond, 2002). This is consistent with our results.

Our results are also different from those of another relevant gambling task study carried out by Breiter and col-

leagues 2001). In their study, increased neural activities were observed in the nucleus accumbens during the period of anticipation. In our study, however, no activation was detected in these regions. This seems to be due to difference in our experimental design from theirs. First, there was a variation in the magnitude of each reward/penalty in their studies as opposed to the consistency in the magnitude of each reward/penalty was in our study. Second, the phase of anticipation toward a reward/penalty and the phase of outcome of a reward/penalty were clearly distinguishable in their task design. On the other hand, we cannot clearly distinguish the brain activities associated with the response to the outcome of a reward/penalty from that associated with the anticipation of a reward/penalty in our task design. Because of the consecutive trials, it was not clear when the anticipation/motivation for a reward/penalty of the next trial starts to occur in our task. The possibility cannot be denied that the reaction to the outcome of a reward/penalty and the anticipation of the next reward/penalty occur together during the second part of each trial as shown in Fig. 1. This is a limitation in our study. For the analyses of our data, however, the onset of hemodyanamic response function was at the point when subjects found out whether the outcome was a win or a loss in each trial. The model was based on our assumption that the subjects' emotional reactions start at that point.

Previous PET studies found that the human orbitofrontal cortex can be activated by monetary reward (Thut et al., 1997). A recent fMRI study also demonstrated that an increase in the activity of the medial orbitofrontal cortex is related to the subjects' receipt of monetary reward and deactivation following penalty (O'Doherty et al., 2001). The authors obtained the converse pattern of activation in the lateral orbitofrontal cortex (activation following penalties, deactivation following rewards). In our data, however, no significant activation was observed in the orbitofrontal cortex in both winning events and losing events. This may be due to the difference in our experimental design from theirs. That is, we did not aim to detect brain regions related to reward or penalty itself, but those regions that are context-dependent in which reward or penalty is received. In our data, however, the results of comparison between all wins and all losses (shown in Table 3) demonstrate the activations in the bilateral ventral striatum, which is consistent with the findings of previous studies (Apicella et al., 1991; Shidara et al., 1998). Another possible reason is that susceptibility and distortion artifacts in the orbitofrontal cortex may have made it difficult to accurately acquire data for this region.

Clinical implications

Although this is speculative, because our study is not a clinical study, we would like to consider the possible clinical relevance of our findings in this paragraph. Taking our results and the results of the previous studies into account, the medial aspect of the prefrontal cortex and the anterior cingulate cortex play a fundamental role in generating and/or regulating appropriate emotional reactions, such as general (i.e., unrelated to reward/penalty) excitement or tension, which are necessary for adjusting oneself to new circumstances. Some clinical studies showed that patients with major depression show a significant decrease in activity of the anterior cingulate cortex (Bench et al., 1993) and hypoperfusion of the anterior cingulate cortex and the medial aspect of the prefrontal cortex (Awata et al., 1998). Patients with posttraumatic stress disorder (PTSD) exhibit a lower anterior cingulate cortex activation in response to disorder-specific stimuli than trauma-exposed normal subjects (Shin et al., 1999). Moreover, patients with major depression or PTSD show abnormality in subjective experience of emotion. Markedly diminished interest or pleasure, complaint of having no feelings, decreased energy or motivation are common symptoms of depression, while anxiety, intrusive memories of the trauma, and increased arousal are major symptoms of PTSD (American Psychiatric Association, 2000). Thus, dysfunction of the anterior cingulate cortex and the prefrontal cortex may lead to failure in regulating mood and in subjective experience of emotion. An emotional or behavioral representation might become inappropriate and result in vulnerability to maladjustment to a new environment. This could be a possible explanation for the observation that the onset of a mood disorder is often induced by both a series of positive life events and a series of negative life events (Holmes and Rahe, 1967; Gelder et al., 2001).

Our data regarding the dorsolateral prefrontal cortex may also have relevance to other mental disoders. Typically, patients with frontal lobe damage show perseverative or "stuck in set" errors in the WCST (Milner, 1963), while schizophrenic patients show a poor performance in the WCST and reduced frontal activation particularly in the right dorsolateral prefrontal cortex in comparison with healthy controls (Riehemann et al., 2001; Wolkin et al., 1992; Volz et al., 1997). This reduction in activation in the right dorsolateral prefrontal cortex is not a pure effect of neuroleptic treatment (Riehemann et al., 2001). These results suggest that the dysfunction of the right dorsolateral prefrontal cortex could be related to the tendency of patients with schizophrenia to show vulnerability to sudden environmental changes (Norman and Malla, 1993).

Conclusion

In conclusion, we demonstrated that there exist brain activities associated with the event-sequence context in which abstract reward or penalty is received. Our data also suggest that information regarding this context is dissociably represented in the human brain, regardless of the nature of event (i.e., reward-related or penalty-related). The anterior cingulate cortex, the medial aspect of the prefrontal

cortex, and the right dorsolateral prefrontal cortex appear to have an important role in these context-dependent activities. Taking the relevant findings about the function of these regions into account, these context-dependent activities are crucial to adapting oneself to new circumstances and may account for the clinical symptoms of various mental illnesses in which dysfunction of these regions has been reported.

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μ-Opioid receptor knockout mice display reduced cocaine conditioned place preference but enhanced sensitization of cocaine-induced locomotion

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Abstract

The μ-opioid receptor (OPRM1) is expressed in brain regions implicated in reward and locomotor processes. Reduced reward, not only from opiates, but also from several other abused substances has been observed in mice with lifelong deletions of the OPRM1 gene. To further define the roles of μ-opioid receptors in psychostimulant actions, cocaine psychomotor stimulant and rewarding effects were examined in wild-type (WT), heterozygous and homozygous μ-opioid receptor knockout mice. While μ-opioid receptor knockout did not affect basal locomotion, locomotor stimulant effects of cocaine were enhanced in a within-subjects dose–response experiment. However, further study revealed that in mice injected with 20 mg/kg for the first time, there was no difference in the locomotor-stimulating effects of cocaine between knockout and wild-type mice. In a sensitization study (modeled after the conditions in the dose–response experiment) although not observed in WT mice, OPRM1 −/− mice did exhibit cocaine sensitization. By stark contrast, and similar to the effects of other rewarding drugs in OPRM1 KO mice, cocaine reward, as assessed by conditioned place preference, was reduced in both homozygous and heterozygous OPRM1 KO mice. The present results confirm a central role of the μ-opioid receptor in drug reward but opposing effects on locomotor sensitization. The reduced cocaine reward identified in heterozygous μ-opioid receptor knockout mice supports the possibility that humans with fewer available μ-opioid receptors might experience less cocaine reward.

Theme: Neural basis of behavior
Topic: Drugs of abuse: cocaine

Keywords: Transgenic mice; Gene knockout; Dopamine; Opiates; Cocaine; Conditioned place preference

1. Introduction

Cocaine is a psychostimulant that inhibits the reuptake of dopamine, serotonin and norepinephrine by blocking their respective transporters, DAT, SERT and NET. Roles for cocaine actions at several transporters in reward mechanisms are supported by recent observations that combined knockouts of DAT and SERT eliminate cocaine conditioned place preference (CPP), while knockout of DAT alone, SERT alone, NET alone, or combined knockout of SERT and NET are ineffective or actually enhance cocaine CPP [23,64,66,77].

Circuits important for cocaine reward and locomotion are not likely to be limited to those using monoaminergic neurotransmitters, however (see [25] for review). Neurons that express preproenkephalin peptides and μ-opioid receptors are present in much of the brain's "reward" and locomotor circuitry, including afferents to the ventral tegmental area and neuronal cell bodies in the nucleus accumbens and caudate/putamen [39,50,68,73,74]. Many authors have reviewed evidence supporting the possibility that these opioidergic systems play roles in the reward elicited by administration of psychomotor stimulants and other abused substances (for review, see Refs. [26,33,41,42,69]). Neurons that express preprodynorphin peptides and kappa opioid receptors are also located in many of these same circuits, although these are often localized in cells that are positioned

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to antagonize rewarding or locomotor effects of preproenkephalin/μ-opioid receptor signaling.

Studies with μ-opioid receptor antagonists and partial agonists implicate roles for these opioidergic circuits in psychostimulant reward [41]. The opioid antagonist naloxone can attenuate the acquisition and expression of cocaine conditioned place preference [20] and reduce cocaine self-administration [34]. Although this agonist is not completely selective for the μ-opioid receptor, its aversive effects appear to be entirely μ receptor-mediated [61]. Similar effects are also seen with the selective μ antagonist naloxonazine [55]. The role of the μ-opioid receptor in cocaine-mediated behaviors may be more complex however, as blockade of many of its effects are more consistently observed with the mixed agonist/antagonist buprenorphine [6,9,10,32,35,38,43–46,60,76], which also seems to have greater anti-cocaine potency in at least some clinical studies [47].

We and others have developed OPRM1 knockout mice that consistently display reduced rewarding and locomotor stimulant effects of μ agonists [1,40,66]. Reward and/or self-administration of other addictive substances is also reduced or eliminated by OPRM1 knockout, as assessed by self-administration and/or conditioned place preference procedures: using ethanol [2,22,57], Δ9-tetrahydrocannabinol [21] and cocaine [2]. The locomotor stimulant effects of an acute cocaine injection were reported to be reduced in OPRM1 knockout mice compared to wild-type controls in one study [78], but unaltered in another [2]. While Yoo et al. [78] demonstrated fewer effects of acute cocaine administration to OPRM1 knockout mice, they identified accelerated sensitization of cocaine-induced locomotion.

We now report assessment of the acute locomotor stimulant effects of cocaine locomotion, sensitization of this locomotor response with repeated administration, and cocaine conditioned place preference in wild-type, heterozygous and homozygous OPRM1 knockout mice that we have developed and extensively characterized [18,24,27,29,37,48,49,51,54,62,63,65,66]. Effects in these knockout mice provide an indication of the surprisingly large, and differential, involvement of these opioidergic systems in cocaine reward and locomotor sensitization. We discuss observations in heterozygous mice that express half of the wild-type complement of μ-opioid receptors in light of the range of human variation in these sites.

2. Methods

2.1. Animals

The OPRM1 KO strain used in this report, in which the first exon of the OPRM1 gene has been deleted, has been described previously [63]. OPRM1 KO mice were generated by +/− × +/− crosses of mice from this strain (>10th generation) producing +/+, +/− and −/− littermates. Mice were genotyped by PCR of tail biopsy tissue. The mice were housed at 24 °C with a 12:12-h light/dark cycle and ad libitum access to food and water. All experiments were conducted on mice between 12 and 20 weeks of age. Both male and female mice were used but sex was not evaluated as a factor in any analyses. There was no obvious evidence for any large sex-dependent differences on these measures. Separate groups of mice were tested for the behavioral effects of cocaine in the conditioned place preference paradigm (N=13–16 mice/genotype/dose), baseline locomotion (N=9–11 mice/genotype), cocaine-stimulated locomotion in a within-subjects dose–response design (N=18–21 mice/genotype), cocaine-stimulated locomotion in a between subjects design (N=11–16 mice/condition), and sensitization of the locomotor stimulant effects of cocaine (N=7–8 mice/condition). All experiments were conducted during the light phase of the cycle, between 9 AM and 3 PM.

2.2. Conditioned place preference

Reward was assessed by conditioned place preference testing using a two compartment Plexiglas chamber [66]. Briefly, one compartment (18 × 18 × 18 cm) had a wire mesh floor (1.3 cm grids) mounted over Plexiglas, and the other compartment (18 × 18 × 18 cm) had corncob bedding on a smooth Plexiglas floor. A removable Plexiglas wall separated the two sides. For pre- and post-conditioning test sessions, a 5-cm opening in the center wall allowed access to both compartments. During the conditioning sessions, the opening was eliminated to restrict animals to a single compartment. Locomotion and time spent in each compartment was recorded using an Optovarimax animal activity monitoring apparatus (Columbus Instruments, Columbus, OH). Conditioned place preferences were assessed by determination of compartment preference in three phases. Initial preference was determined as the side in which a mouse spent more than 600 s out of a 20-min trial. Conditioning was conducted over a 2-day period in which cocaine hydrochloride (0, 5 or 10 mg/kg, s.c.) was administered to the animal when it was confined for 30 min in the initially non-preferred compartment and saline administered to the animal when restricted for 30 min to the initially preferred compartment. Animals received two conditioning sessions per day, counterbalanced between saline and cocaine for a total of two saline pairings and two cocaine pairings (or four saline pairings). Conditioned place preference assessment followed the last conditioning session and the last injection by 24 h. Time spent in the drug-paired compartment was compared to the pre-conditioning values obtained on initial assessments.

2.3. Locomotor testing and basal locomotion

Locomotor activity was assessed as total distance traveled. Distance was calculated from measurement of infrared

beam breaks by mice placed individually in 46 × 25 × 19 cm clear plastic cages in Optovarimax activity monitors (Columbus Instruments) under dim light, sound-attenuated conditions. An initial 2-h activity test was conducted in +/+, +/− and −/− OPRM1 mice under novel conditions.

2.4. Cocaine-stimulated locomotion in a within-subjects dose–response design

A separate set of mice were tested for cocaine-stimulated activity using a within-subjects design. After habituation to the apparatus for 1 h mice were injected with cocaine hydrochloride (0, 5, 10 or 20 mg/kg, s.c.), and distance traveled was monitored for an additional 2 h. Doses were counterbalanced across testing days, and at least 48 h separated each testing day. This paradigm, with substantial pre-drug habituation, is commonly used to examine the dose–response characteristics of stimulant drugs because it does not normally produce locomotor sensitization. However, with this possibility in mind two further experiments were conducted.

2.5. Cocaine-stimulated locomotion in a between-subjects design

A separate set of mice was tested for cocaine-stimulated activity using a between-subjects design. Because cocaine did not produce substantial locomotor stimulation in WT mice of this strain, and differences were only observed at the highest doses in the previous experiment (see below), only the effects of cocaine and saline were examined. In each case, each subject was tested only once. After habituation to the apparatus for 2 h mice were injected with either cocaine hydrochloride (20 mg/kg, s.c.) or saline, and the distance traveled was monitored for an additional 2 h.

2.6. Sensitization of locomotor activity

Within-subjects dose–effect curves for locomotor activity are open to the possible confounding effects of locomotor sensitization. Although the within-subjects design utilized in this study minimizes such effects through long habituation prior to drug administration, differences between genotypes observed in within-subjects locomotor testing might possibly reflect differences in locomotor sensitization rather than naive sensitivity to the locomotor-stimulating effects of cocaine. To assess this possibility, separate groups of mice were assessed for sensitization of locomotor activity using a paradigm that closely parallels that used in the dose–response study. Mice were placed in the locomotor testing apparatus as described above each day for 5 days. After 1 h of habituation, mice were injected with either cocaine (20 mg/kg s.c.; this dose was chosen because it was thought that it would produce the maximal amount of sensitization under these conditions) or saline (1 ml/kg) and monitored for 1 h, so that mice were injected five times with cocaine or five times with saline and locomotor responses were assessed.

2.7. Drug

Cocaine HCl (NIDA IRP Drug Supply Program) was dissolved in saline and administered s.c. in a volume of 1.0 ml/kg.

2.8. Statistical analyses

Conditioned place preference was calculated as the difference in time spent on the non-preferred side post-conditioning and the time spent on the non-preferred side pre-conditioning. These data were then subjected to ANOVA with the between subjects factors of GENOTYPE and DOSE.

Baseline locomotor activity data were analyzed by ANOVA with the between subjects factor of GENOTYPE and the within-subjects factor of TIME, with the data divided into 10-min bins. For cocaine-stimulated locomotion the distance traveled was summed over each test session and analyzed by ANOVA with the between subjects factor of GENOTYPE and the within-subjects factor of DRUG (cocaine versus saline). The total locomotor activity after cocaine or saline administration were summed for analysis of sensitization data using the between subjects factors of GENOTYPE and DRUG (cocaine versus saline) and the within-subjects factor of DAY.

Post hoc comparisons were done using Scheffe's test for comparison of two means or by post hoc one-way ANOVA for comparison of dose effects for each GENOTYPE.

3. Results

3.1. Cocaine conditioned place preference

Both doses of cocaine increased preference for the initially non-preferred compartment (Fig. 1; DRUG: $F[2,127]=17.5$, $p<0.0001$). Preference for the compartment associated with either dose of cocaine was reduced to about half of wild-type values in both in OPRM1+/− and OPRM1 −/− knockout mice at both doses of cocaine (Fig. 1; GENOTYPE: $F[2,127]=6.8$, $p<0.01$). The effect of cocaine in OPRM1 −/− mice at the 20 mg/kg dose was not significantly different from saline, and these mice had a significantly reduced preference for the cocaine paired side compared to wild-type littermates ($p<0.05$, Scheffe's comparison).

3.2. Baseline locomotion

All mice exhibited normal elevation of locomotor activity in a novel environment and subsequent habituation of locomotor activity over the period of testing. However,

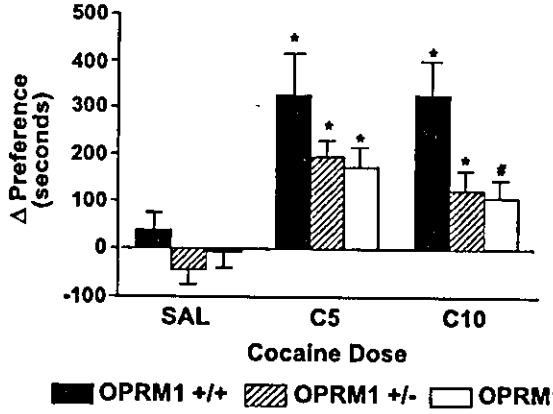

Fig. 1. The conditioned place preference for cocaine is reduced in OPRM1 KO mice. Conditioned place preference induced by cocaine (5 or 10 mg/kg, s.c.; C5 and C10) in OPRM1+/+, OPRM1+/− and OPRM1 −/− mice expressed as the difference between the pre-conditioning preference and the post-conditioning preference. Data expressed as mean ± standard error of the mean. *Significant difference from saline treatment ($p<0.05$, Scheffe's post hoc comparison). #Significant difference from wild-type mice ($p<0.05$, Scheffe's post hoc comparison).

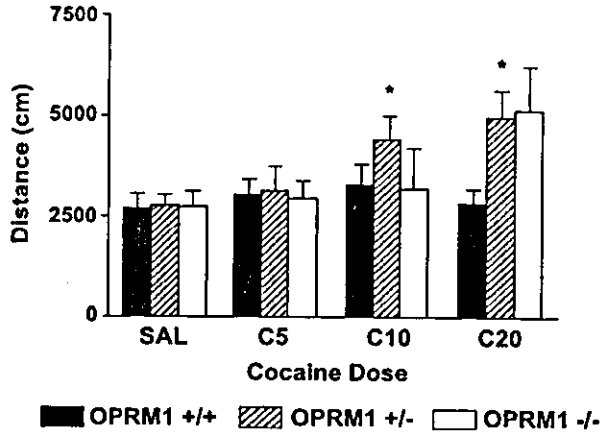

Fig. 3. Within-subjects dose–response study of cocaine-induced locomotor activity in OPRM1 KO mice. Cocaine-induced locomotor activity in OPRM1+/+, OPRM1+/− and OPRM1 −/− mice expressed as the total distance traveled over 2 h of testing after the injection (0, 5, 10 and 20 mg/kg cocaine HCl s.c.: SAL, C5, C10 and C20, respectively). Data expressed as mean ± standard error of the mean. *Significant difference from saline based on Scheffe's post hoc comparison ($p<0.05$).

baseline locomotion, tested over a period of 2 h, was not affected by GENOTYPE (Fig. 2; $F[2,29]=0.1$, ns).

3.3. Cocaine-stimulated locomotion in a within-subjects dose–response design

Locomotion during habituation periods prior to drug treatments was not affected by GENOTYPE (data not shown; $F[2,58]=0.1$, ns). Cocaine produced modest but significant locomotor increases that were dependent on DOSE (Fig. 3; $F[2,58]=4.5$, $p<0.01$). Wild-type mice of this strain, of mixed background from the C57/B6 and 129SV progenitor strains, displayed no significant effect of cocaine DOSE (post hoc ANOVA on OPRM1+/+ mice alone: $F[3,62]=0.7$, ns), while a significant effect of cocaine DOSE was observed for OPRM1+/− mice (post hoc ANOVA on OPRM1+/− mice alone: $F[3,51]=3.5$, $p<0.03$) and a trend toward significance was noted in −/− mice (post hoc ANOVA on OPRM1 −/− mice alone: $F[3,60]=2.4$, $p<0.08$).

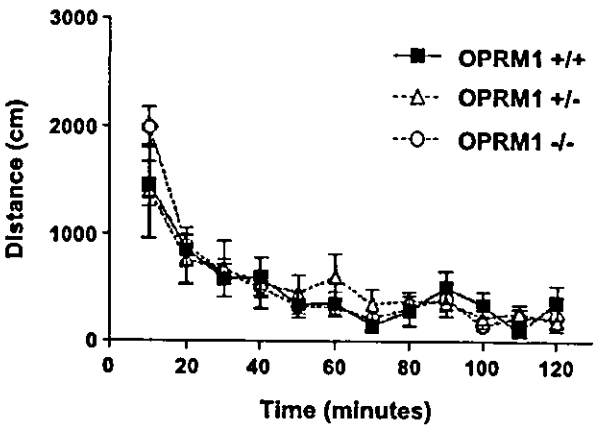

Fig. 2. Time-course of locomotor habituation to a novel environment in OPRM1 KO mice. Time-course of locomotor activity in OPRM1+/+, OPRM1+/− and OPRM1 −/− mice expressed in terms of total distance traveled over the 2-h session. Data expressed as mean ± standard error of the mean.

3.4. Cocaine-stimulated locomotion in a between-subjects design

The locomotor response to cocaine was more robust in this experiment, producing a significant effect of DRUG in

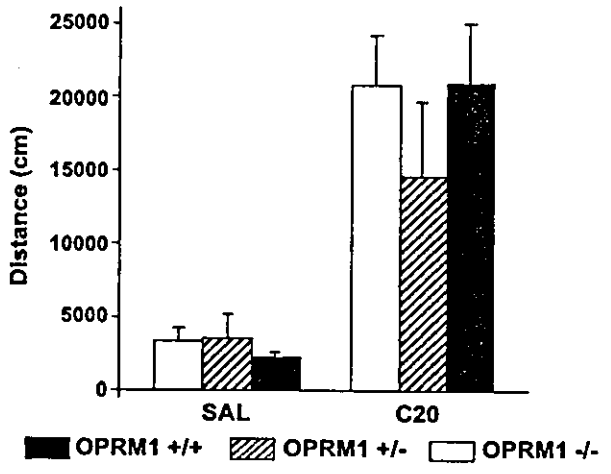

Fig. 4. Between-subjects study of cocaine-induced locomotor activity in OPRM1 KO mice. Cocaine-induced locomotor activity in OPRM1+/+, OPRM1+/− and OPRM1 −/− mice expressed as the total distance traveled over 2 h of testing after the injection (0 and 20 mg/kg cocaine HCl s.c.: SAL, C20, respectively). Data expressed as mean ± standard error of the mean.

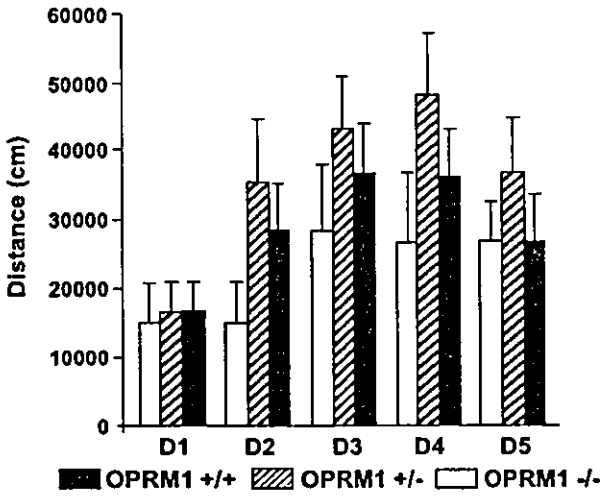

Fig. 5. Sensitization of cocaine-stimulated locomotion in OPRM1 KO mice. Cocaine-induced locomotor activity in OPRM +/+, OPRM +/− and OPRM −/− mice expressed as the total distance travelled over 2 h of testing after cocaine injection (20 mg/kg cocaine HCl s.c.) on five consecutive days (D1–D5). Data expressed as mean ± standard error of the mean.

all groups (Fig. 4; $F[1,73]=35.4$, $p<0.0001$) but there was no effect of GENOTYPE ($F[2,73]=0.4$, ns).

3.5. Sensitization of cocaine-stimulated locomotion

Under the conditions used in this experiment, cocaine produced substantial locomotor activation (Fig. 5; DRUG, $F[1,45]=51.2$, $p<0.0001$) and there a was significant increase in this locomotor response to cocaine with repeated cocaine administration (DAYS, $F[4,164]=8.3$, $p<0.0001$). However, there was no significant effect of GENOTYPE or a GENOTYPE × DAYS interaction as determined by ANOVA. As in the prior experiment, but in contrast to the within-subjects experiment, there was no effect of GENOTYPE at this dose of cocaine (20 mg/kg) on the first day of administration. Although the ANOVA did not reveal any significant differences, individual post hoc ANOVA of each genotype to determine which genotypes contributed to the significant overall effect of DAYS found significant effects in OPRM1+/− (DAYS, $F[4,56]=4.6$, $p<0.003$) and OPRM1 −/− (DAYS, $F[4,56]=4.2$, $p<0.006$) mice, but not OPRM+/+ mice (DAYS, $F[4,164]=1.6$, ns).

4. Discussion

Lifelong deletion of all or half of the brain's normal complement of μ-opioid receptors reduces the rewarding effects of cocaine in a conditioned place preference paradigm. Findings in heterozygote mice fit well with previously reported data from experiments using doses of opioid antagonists likely to block many, but not all, μ receptors. We discuss these findings in comparison to findings with other drugs, contrasting effects on locomotion and sensitization of locomotion, caveats, implications of the findings for interactions between monoaminergic and opioidergic circuitries controlling reward and locomotor function, and the relevance of these observations for possible effects of human individual differences in μ-opioid receptor expression on psychostimulant reward and locomotion.

The magnitude of the effects of μ-opioid receptor reduction or deletion on reward forms a coherent picture of the role of these receptors in the rewarding effects of multiple classes of abused substances. Deletion of μ-opioid receptors eliminates the conditioned place preference produced by opiates such as morphine, while heterozygous μ opiate receptor knockouts display reduced but significant morphine place preferences [40,66]. These findings have been taken as confirmation that most or all of the rewarding effects of morphine administration depend on its agonist actions at μ-opioid receptors. Most of the place preference for the active cannabinoid, THC, is also eliminated in OPRM1 knockout mice [21]. There are more modest but still significant effects of OPRM1 knockout on conditioned place preference for ethanol [22] and cocaine (Ref. [2] and the present results). Circuits bearing μ-opioid receptors thus seem likely to be crucial for morphine's rewarding actions, highly important for the reward from marijuana's active ingredient, and of significant importance for alcohol and psychostimulant reward. Taken together, these lines of experimental evidence support the idea that brain circuits containing μ-opioid receptors, which may use preproenkephalin peptides as their major endogenous agonists, play a central role in drug-induced reward processes. These data contrast with those from CB1 cannabinoid receptor gene knockouts. These knockouts eliminate the rewarding effects of cannabinoids [36], opiates [13,14,36] (but see Ref. [56]), and nicotine [7] (but see Ref. [13]), but leave the rewarding effects of psychostimulants intact [13,14].

Such a central role for μ-opioid receptors in drug reward does not necessarily mean that this role is limited to the μ-opioid receptors expressed in only a single cell group. Reward can be elicited by opiates microinjected into a number of brain regions [3,4,8,15,16,52]. Reward from systemically administered opiates can be blocked by local injection of opiate antagonists into a number of brain regions [5,11,12,71,72]. Based on this evidence μ-opioid receptors located on afferents to the ventral tegmental area, cells of the nucleus accumbens and caudate/putamen, and even cells and afferents to amygdaloid subnuclei are candidates for roles in μ-opioid receptor-dependent circuits important for reward from each of these substances [39,50,68,73,74]. Several of these nuclei could also be sites for dopamine–opioid interactions. Electron microscopic immunohistochemistry has shown that some single striatal neurons receive both dopaminergic and μ-opioidergic inputs [75], for example. Interaction between dopaminergic and μ-opioidergic neurons in the ventral tegmental area (VTA) allows μ-opioid receptor-mediated